

Preface

The book covers a critical compilation of analytical methods used for the monitoring of pesticides and their degradation products in water. The broad range of analytical methodologies includes conventional techniques routinely used for the trace determination of pesticides in water as well as advanced monitoring techniques developed during the last few years. This book contains up-to-date material that we have been lecturing in recent years for short courses entitled: "Sample handling of pesticides in the aquatic environment" and it is the direct result of our experience in the field of pesticide analysis. The content of the book should solve most of the problems encountered in pesticide analysis, both for newcomers and expert laboratories looking either for a multiresidue analysis or for a tailor-made determination of a pesticide and/or a polar metabolite.

The book is structured in six chapters, beginning with general aspects of pesticides such as usage, physicochemical parameters and occurrence in the environment. The second chapter is devoted to sampling from water matrices, stability methods of pesticides in water using advanced protocols such as solid phase extraction materials and quality assurance issues. The general chromatographic methods for pesticides are reported, including the newly developed electrophoresis methods. GC-MS and LC-MS are mainly used as confirmatory analytical methods but their increasing use as quantitative analytical techniques is also emphasized. Sample preparation methodologies, including off-line and on-line techniques, are described in the next two chapters, with a comprehensive list of examples of pesticides and many metabolites, including the use of different GC-based methods and LC-based-methods. The final chapter is devoted to the development of biological techniques that can be used in most cases without any sample pre-treatment and so are useful as early warning methods. The use of immunoassays, also called ELISA, which have been widely applied in monitoring programs, mainly in the US and scarcely in Europe, and biosensors, are described and a broad list of examples from the recent literature is reported.

Overall, the book answers one of the key problems in pesticide analysis: the diversity of chemical functional groups with varying polarity and physicochemical properties. Pesticides and their metabolites have received particular attention during the last few years in environmental trace organic analysis. For instance, in the case of ground water, the use of pesticides has become a cause for concern and can move through the soil into ground water, a phenomenon once thought improbable. Water soluble pesticides and their polar metabolites are generally transported to estuarine

and coastal waters. Estuarine waters feature gradients of both pollutant concentrations and physicochemical characteristics such as salinity, turbidity and pH, and all these parameters must be carefully considered when developing methods of analysis for trace organics in estuarine waters.

Environmental sampling of pesticides in water has also been discussed in detail. The various protocols and devices needed for sampling sea water samples, usually using large sample volumes of more than 50 l either with LLE or SPE, with the problems encountered due to dissolved and particulate matter, are different from those derived for drinking water and well water sampling.

A major emphasis of the book concerns the various aspects related to the sample preparation of pesticides and their metabolites from water matrices. This question has been recognized to be a bottleneck and it has traditionally been neglected in the literature. We should comment, following the late R.W. Frei's comments, that the most sophisticated hardware is useless if the chemistry in the protocol does not work. During the last few years new sorbent materials have appeared – carbon type, polymeric sorbents with high capacity and immunosorbents – which can more efficiently trap highly polar compounds.

The book covers advanced automation methods based usually on solid phase extraction techniques; PROSPEKT, OSP-2 and ASPEC XL are examples of commercially available equipment that are of growing importance. These systems are generally coupled to LC and GC techniques and in recent years more and more applications have been published using either GC-MS and/or LC-MS, that serve to avoid false positive determinations.

Biosensors and immunoassays are also of growing interest for the determination of pesticides in environmental matrices and are covered in the last chapter of this book. The rapid development of these techniques, which are more sensitive and can work in drastic environmental conditions, such as very different pH and salinity values, makes them very useful and complementary to conventional GC and/or LC techniques for the trace determination of pesticides.

Finally the authors wish to express their gratitude to a number of collaborators who have participated in the compilation of information and who are aware of all the work in finalizing the present book. Without them such a book project could not be successfully completed. They include Ph.D. colleagues V. Pichon, J. Dugay, S. Lacorte and S. Chiron, former Ph.D. students S. Guenu, S. Dupas, L. Chen, C. Cau-Dit-Coumes, M. Honing, G. Durand and the late C. Molina and present Ph.D. students A. Oubiña, J. Gascon, J.S. Salau, G.A. Peñuela, D. Puig, I. Ferrer, J. Riu, M. Castillo, E. Martinez and E. Mallat and the technicians R. Alonso and R. Chaler. Our thanks to them all.

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CHAPTER 1

Pesticides and their Degradation Products: Characteristics, Usage and Environmental Behaviour

1.1. INTRODUCTION

Since the late 1970s, concern about the contamination of water has increased, partly due to the increasing number of pesticide detections in water but also partly in connection with the strict directives that were set up for protecting drinking water sources, especially in North America and Western Europe. Pesticide residues have been monitored with regard to regulatory compliance at levels that can exceed the maximum allowed concentration in surface, groundwater and finished drinking waters [1–3]. The detection of some pesticides, mainly herbicides, in water sources has also indicated a lack of understanding of their behaviour and was the stimulus for many studies on their fate and transport. With the improvement of analytical methods involving gas and liquid chromatography coupled with mass spectrometry, the use of new sorbent materials with higher trapping abilities, and the development of automated methods, several degradation products have been identified in recent years. The term degradation products in this volume refers to both biotic and abiotic transformation products of a parent pesticide and therefore includes metabolites.

1.1.1. Historical context of pesticide use

Although detailed evidence of water contamination is rather recent, the use of pesticides began several decades ago. The commercial active ingredients were discovered incidentally during global biological tests on a great number of compounds [4]. The use of synthetic organic pesticides began in the early 1930s after the demonstration of the insecticidal properties of some alkyl thiocyanates and fungicidal properties of dithiocarbamates. However, the real beginning of the pesticide industry occurred with the introduction of DDT, patented in 1942; it remained one of the leading insecticide products for during two decades. During the same period, the first herbicide 2,4-D was introduced into the market in 1944. In the period 1945–1955,

the so-called “second generation of pesticides”, i.e. most of the organophosphorus insecticides, many carbamates and ureas, was developed, some representatives of which are still commonly used today. Quaternary ammonium materials and triazines were introduced in the years 1955–1960.

In the period 1960–70 many fungicides were developed, such as benzimidazoles, pyrimidines, triazoles and imidazoles. The last two groups act as inhibitors of sterol biosynthesis and represent a large part of the fungicide market. In the same period, major concern about pesticide risk appeared as a result of ecotoxicological effects following the intensive use of DDT and other persistent organochlorines, which led to the banning (DDT in 1972 in the USA) or the severe restriction of these compounds. They were gradually replaced by organophosphorus and carbamate compounds.

The third generation of pesticides appeared in the period 1970–1980. It includes pyrethroids and sulfonyleureas, which can be used at low dosage rates because of their strong biological action. Sterilants, pheromones, and chitin inhibitors have appeared more recently.

1.1.2. Trends in the world usage of pesticides

The assessment of distribution and effects of pesticide residues in the environment requires a consideration of the usage of pesticides which were applied in the past and at the present. Data on pesticide usage and the amounts of active ingredients are seldom complete, but information on the agrochemical market is more readily available.

Economic figures on pesticides (herbicides, insecticides, fungicides and others) on the market during the period 1960–1993 have been published elsewhere [5,6] and are shown in Table 1.1. It is shown that the pesticide market is an important one with an average 12% growth in dollar terms. One notable trend has been in the increased use of herbicides and the relative decrease of fungicides. In 1993, the total volume of active ingredients was estimated to be 2000 million tons, with a ratio of 57% of herbicides, 23% of insecticides and 12% of fungicides. The increase in the amounts of ingredients employed depends on geographic areas. In USA and Europe it has remained roughly constant since 1980. This is explained by lower application rates resulting from the introduction of more potent pesticides, more efficient use, and increasing concern of farmers and agencies in developing plans to reduce the risk and the use of pesticides. In the coming years, an increase in pesticide use is expected to occur rapidly in developing countries whereas a slight reduction will continue in North America and Western Europe.

Table 1.2 indicates the top ten pesticides used in the world, in the USA, and in Europe. These top ten herbicides are not common to all areas and some trends can be seen. First, there are common herbicides, such as atrazine and glyphosate, that are used worldwide. Secondly, several herbicides have high world usage, but not in Europe and the USA. This is true for propanil, thiobencarb and trifluralin; these her-

TABLE 1.1
TRENDS IN THE PESTICIDE WORLD MARKET AND PERCENTAGES OF ACTIVE
INGREDIENTS BY USAGE. FROM REFS. [5,6]

	1960	1970	1980	1993
<i>World conventional pesticide sale in millions of dollars</i>				
Total pesticides	580	2700	11600	25300
<i>Percentage of the total market value for the year for each pesticide usage</i>				
Herbicides ^a	19	35	42	45
Insecticides ^b	37	38	35	31
Fungicides ^c	40	22	18	19
Others ^d	4	5	5	5

Conventional pesticides represents those used in agricultural and non-agricultural sectors, as well as those use in private gardens and home. They exclude wood preservatives and disinfectants.

^aIncludes plant growth regulators.

^bIncludes miticides and contact nematicides.

^cInclude sulfur but not wood preservatives.

^dInclude rodenticides, fumigants and molluscicides, but not wood preservatives and disinfectants.

TABLE 1.2
TOP TEN HERBICIDES USED IN THE WORLD [3], IN THE USA [2] AND IN EUROPE [1]

Herbicides	World usage	USA	Europe
Glyphosate	x	x	x
Alachlor	x	x	
Metolachlor	x	x	
Thiobencarb	x		
Paraquat	x		
2,4 D	x	x	
Atrazine	x	x	x
Propanil	x		
MCPA	x		x
Trifluralin	x	x	
Butylate		x	
EPTC		x	
Pendimethalin		x	
Cyanazine		x	
Isoproturon			x
Chlorotoluron			x
MCPP			x
Maneb			x
Metam-sodium			x
Mancozeb			x
1,3-Dichloropropene			x

bicides are associated with rice culture which is the most extensive cultivation in the world and the basis of food for China and many other developed countries. The other interesting point in Table 1.2 is that there are some differences between the top ten herbicides used in Europe and the USA. Whereas in Europe the urea herbicides are used extensively and contaminate many water sources, they are not in the top ten list for the USA. It can be observed in the present book and in many published papers that many of the pesticides indicated in the top ten list are detected in ground- and surface waters around the world. The absence of several of these compounds, e.g., glyphosate and paraquat, in pilot monitoring surveys, can have two reasons. First, the ionic structure of these molecules means they can easily be bound to the inorganic component of the soil. These pesticides are commonly defined as practically immobile and non leaching. Questions arise from all the monitoring data that in several cases do not match the usage data. It is difficult to define whether the most frequently detected pesticides are really the most commonly occurring in the environment, or whether they are just most frequently investigated. It is difficult to be sure whether compounds currently unreported really are not present in water or whether they are not sought, for reasons such as analytical difficulties, or low public concern. The significance of non detection could have different meanings, depending on the detection limit of the technique used. In this respect a factor of two between the detection limits in the USA, Europe and less developed countries can be easily found. The question of the analytical difficulties in determining pesticides in water sources is certainly true for glyphosate and paraquat. The analytical methods for analyzing these pesticides are tedious and complicated, and many official laboratories do not have the techniques. Generally, the most common methods involve either liquid-liquid extraction or solid phase extraction followed by some kind of gas chromatographic detection. In this way, compounds such as atrazine, alachlor and metolachlor have been detected frequently, because practically all the methods can detect them. Considering all the points indicated above, the usage data give an indication and trends in the amounts in which compounds are being released into the environment, and in each case the analytical techniques should be adapted for detecting the priority- and widely used- pesticides in the environment. In several cases, as for glyphosate and paraquat, this will require tailor-made analytical methods.

Herbicides represent economically and quantitatively the most important category and they have shown the highest average annual increase between 1960 and 1993 [7]. Figure 1.1 shows the regional use of agrochemicals, based on the market values of 1993 [6]. The geographic distribution of pesticide usage depends on the culture. At present, more than 80% of the world market of herbicides is concentrated in the three geographic areas where agriculture is the most developed: North America, Western Europe and East Asia. Insecticides are employed most in North America, East Asia and Latin America, and it is estimated that the half of the world-use of insecticides is in developing countries, especially in tropical regions. In China, a major

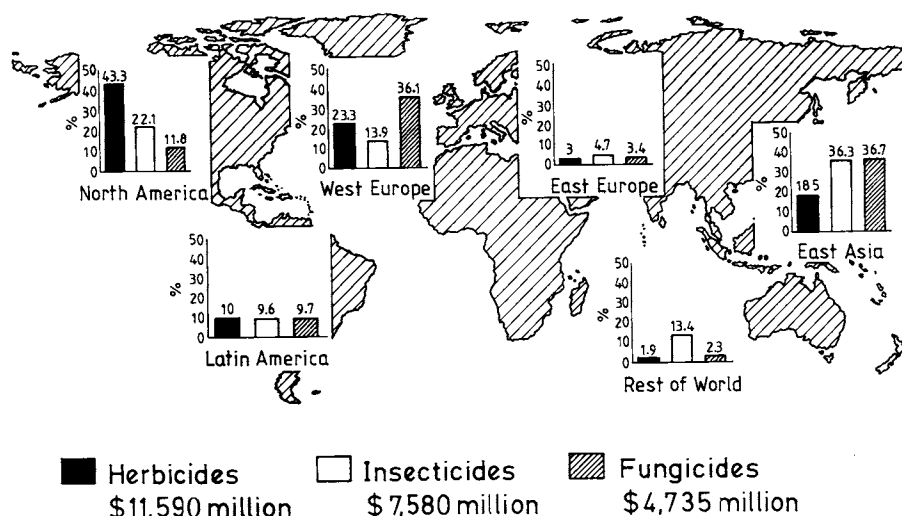


Fig. 1.1. Regional use of pesticides, based on market values, 1993. From Ref. [6] with permission.

world producer of rice and wheat, the total consumption of pesticides in 1990 includes 70% of insecticides, mainly organophosphorus [8].

Contamination of water by pesticides and their degradation products depends on several physico-chemical, agricultural and environmental factors. The first information to be considered for the leaching of a compound in a certain area regards its use. National and regional reports, when available, can then provide specific information. Table 1.3 reports the leading active ingredients which were sold in 1993 in the USA, and Table 1.4 lists the pesticides which were sold above 500 tons per year in 1990 in Western Europe. Only some leading compounds are common to the two lists, but it is easy to understand that both the types of culture and the influence of the pesticide industry may be different. It is worthwhile to note the importance of herbicides (see

TABLE 1.3
PESTICIDES MOST COMMONLY USED IN THE US AGRICULTURAL CROP PRODUCTION IN 1993 [5]

Range: 13–30 (millions of kg)	Range: 9–13 (millions of kg)	Range: 4–9 (millions of kg)	Range: 1.5–4 (millions of kg)
Atrazine	2,4-D	EPTC	Bentazone
Metolachlor	Metam sodium	Chlorpyrifos	Mancozeb
Sulfur	Trifluralin	Chlorothanil	Copper hydroxide
Alachlor	Petroleum oil	Propanil	Parathion
Methyl-bromide	Pendimethalin	Dicamba	Simazine
Cyanazine	Glyphosate	Terbufos	Butylate
Dichloropropene			

TABLE 1.4
PESTICIDES USED IN LARGE AMOUNTS IN WESTERN EUROPEAN COUNTRIES (ADAPTED
FROM REF. [1])

>2000 tons/year	1000–2000 tons/year	500–1000 tons/year
Atrazine	Alachlor	Azinphos-ethyl
1,3-Dichloropropene	Chlortolurone	Azinphos-methyl
Glyphosate	Chlormequat	Bentazone
Isoproturon	Chlorothalonil	Captan
Mancozeb	2,4-D	Carbetamide
Maneb	Dichlorprop	Carbendazim
MCCP (Mecoprop)	1,2-Dichloropropane	Chloridazon
MCPA	Dimethoate	Metazachlor
Metam-sodium	Ioxynil	Metolachlor
	Malathion	Molinate
	Metamitron	Paraquat
	Methabenzthiazuron	Phorate
	Methyl bromide	Pendimethalin
	Parathion	Propanil
	Prochloraz	Propiconazole
	Trichloroacetic acid	Propineb
	Trifluralin	Simazine
		Tri-allate
		Tridemorph
		Zineb
		Ziram

also Table 1.2.), which have been shown to be the most common contaminants in surface water. Atrazine, metolachlor, alachlor, cyanazine and 2,4-D are used in the largest amounts. Degradation products of atrazine, cyanazine and alachlor have frequently been detected along with the parent molecules in recent surveys performed by the US Geological Survey [9,10].

1.1.3. Non-agricultural uses of pesticides

A further source of pollution of ground waters is the non-agricultural use of pesticides. Most of the non-point source pollution of ground- and surface waters by pesticides is of agricultural origin (e.g., non-irrigated, irrigated or speciality crop production, pasture land, gangland) or land-disposal origin (landfills, channelization, hazardous wastes, or flow regulation). However, in recent years particular attention has been devoted to the non-agricultural uses of pesticides, e.g., on highways and railroads, and on golf courses. The non-agricultural uses of pesticides accounted for 550 tons of active ingredient in 1989, in England and Wales alone, with atrazine, simazine and diuron being the three major compounds used [11]. A considerable list of professional and consumer end-users of a wide variety of pesticides has been re-

ported [12]. The list includes application in grass-management (golf courses, educational facilities, parks, and cemeteries), in industrial vegetation control (industrial facilities, electric utilities, roadways, railroads, pipelines), in public health (mosquito-abatement districts, rodent-control areas, and aquatic areas) and for non-agricultural crops such as commercial forestry and horticulture and plant-nurseries). Considering all these aspects of non-agricultural pollution, probably one of the most dangerous for ground water pollution relates to golf courses. In the US alone, there are over 14 000 golf courses; assuming an average of 48.6 ha per course would mean that there are over 0.68 million ha of turf-grass in the golf course industry. The number of golfers in the US alone is estimated to be 22 million, and by the year 2000 the number of players could easily exceed 30 million. To keep up with the demands of the rapidly increasing number of golfers, it is suggested that a new golf course must be opened every day over the next few years [13]. Pesticides being used on golf courses, and for which studies on pesticide movement were carried out, are dicamba, mecoprop (MCP), chlorothalonil, chlorpyrifos and pendimethalin. The compounds are used for the control of crabgrass in turf-grass (including greens) and include metalaxyl, glyphosate, trichlorfon, 2,4-D, MCP, chlorpyrifos, chlorothalonil, isofenphos and dithiopyr [14–16]. The reported data indicate that less than 1% was transported in the leachate from the base of the lysimeters. High concentrations of 200–800 $\mu\text{g/l}$ of the treatment herbicides, were detected in the runoff water from treated simulated fairway plots.

In the above studies a risk assessment evaluation was carried out, using the DRASTIC model and GUS index. The non-agricultural use of pesticides has also encouraged comparative studies; one reported for chlorpyrifos showed the fate of this compound to be similar in both urban and agricultural environments. The high application rate of chlorpyrifos in the urban environment (1000 ppm for termite control versus 10 ppm for agricultural use) meant that in the urban environment it had a longer residual control and also a slower degradation resulting from the elevated concentrations [15].

Non-agricultural use of pesticides (e.g., for weed control on roads, railroads, paths, industrial and recreational areas, and for aquatic- and public-health pest control) is potentially a significant threat to ground- and surface water. This is especially true because such use involves application to areas where possible fast routes to ground water exist by runoff. Information on this is difficult to find, because it is not often collected. However, the amount of pesticides used for non-agricultural purposes is not negligible. Data available in the USA from the year 1993 are given in Table 1.5. The non-industrial applications and private home and garden consumption together represent about 25% of the total volume, and this ratio has been about the same for the last 15 years. Insecticides and fungicides represent 31 and 35% of the total consumption, respectively. Leading products in the US are in order of decreasing amounts: 2,4-D, chlorpyrifos, diazinon, glyphosate, malathion, dicamba, diuron, naled, MCP and carbaryl [5]. In Europe, usage varies with the country [1]. In the

TABLE 1.5
VOLUME OF CONVENTIONAL PESTICIDE ACTIVE INGREDIENTS IN USAGE SECTOR IN
1993, EXPRESSED AS PERCENTAGE (FROM REF. [5])

	Agricultural use	Industrial, commercial and government	Home and garden
Herbicides (%)	78	18	4
Insecticides (%)	69	18	13
Fungicides (%)	64	27	8
Others (%)	90	8	4
Total (%)	75	18	7
Total (millions of kg)	265	89	33

UK, the following account for 88% of the total estimated non-agricultural usage: atrazine, simazine, diuron, 2,4-D, mecoprop, amitrole, glyphosate, sodium chlorate and MCPA. In The Netherlands, dalapon, dichlobenil and chlorthiamid should be added to that list. In France, diuron and amitrole represented more than 50% of pesticides applied on railways in 1988–1989. In Spain, diuron, glyphosate, simazine and paraquat were the main non-agricultural products in 1990.

The agricultural and non-agricultural uses of pesticides represent a large number of different active ingredients. The usage of pesticides in 1993–1994 involved about 21 000 pesticide products and 860 active ingredients registered under the Federal Pesticides Law of the US. The last edition of the Pesticide Manual (1994) contains 725 “main entries”, corresponding to chemicals and biological agents used as active ingredients which are in use or being developed, and 559 “superseded entries” which correspond to materials believed to be no longer manufactured or marketed for crop protection use [17].

1.1.4. Formulations

Pesticides are generally sold and applied in agriculture as formulations. The importance of efficient delivery to the target site of a herbicide's activity is generally recognized. Delivery of a potential dose of active ingredient depends upon a complex interaction of factors including the efficiency of application, absorption, translocation, immobilization, and detoxification. Formulations of a pesticide should be as effective as possible and generally contain a mixture of surfactants, mineral and vegetable oils, emulsifiers, and salts, which are considered with particular reference to the penetration of the cuticle by foliage-applied herbicides. There are few requirements in the European Community for non-pesticidal co-formulants and adjuvants. For example information should be given on: (i) acute toxicity data via oral and dermal routes; (ii) skin and eye irritancy data and skin sensitization data; (iii) formulation fish toxicity data; and (iv) residue data on appropriate crops.

The adjuvant is a specific substance, other than water, which itself has no significant pesticidal properties, but which enhances or is intended to enhance the effectiveness of a particular pesticide to which it is added. It can only be used with a pesticide in accordance with the conditions of approval of that pesticide. A list of authorized adjuvants is given in Tables 1.6 and 1.7.

Among the adjuvants listed, there are many surfactants; their function is as spreaders, stickers, antifoamers, compatibility agents, or activators. Surfactants play an important role in the formulation of herbicides, as emulsifiers for emulsifiable concentrates and microemulsions or as wetting and dispersing agents for wettable powders, water dispersible granules and suspension concentrates. They can also be used as spray-tank additives to enhance their adhesion and wetting of foliage and uptake into the plant. Four classes of surfactants have been defined: anionic, cationic, non-ionic and amphoteric. Surfactants have a major effect on the surface tension of the spray droplets at the air–water interface and on the contact angle at the water–plant interface. They can also influence the spray droplet spectrum, spray drift, and the efficiency of delivery to the leaf surface. The adhesion, spreading, wetting, coverage and run-off may all be affected. Surfactants can be divided into a number of broad categories. Spray modifiers are added to improve the wetting and spreading properties of formulations and are regarded as more important for non-systemic herbicides, which need to be applied uniformly to the target canopy for maximum effect. Another type, the so-called activator surfactants, is added specially to enhance the foliar absorption of systemic herbicides, thereby enhancing their ultimate biological activity. Surfactants commonly used as activator adjuvants include polyoxyethylene condensate, with alcohols, alkylphenols, substituted sorbitants, and alkylamines with hydrophilic-lipophilic balance values ranging from 10 to 123. These products are normally used at final concentrations of 1–5 mg/l in the spray solution.

Oil based adjuvants are used for a variety of reasons, such as reducing vapour-loss of herbicide, enhancing the performance of herbicides on recalcitrant species, and in adverse conditions. Traditionally, spray formulations have incorporated petroleum-based oils, but more recently, oils extracted from crop seeds such as soybean, sunflower, canola and coconut have been used. In general, petroleum oil adjuvants enhance the performance of foliage-applied herbicides by increasing the absorption of the active ingredient. Such adjuvants consist of a mixture of phyto-bland oil and emulsifiers, and their effectiveness is related to the chemical composition of the oil. Their ability to form a stable oil-in-water emulsion depends upon the pesticide and the application equipment; the emulsion must be sufficiently stable to disperse the active ingredient and oil uniformly in the spray mixture and thus to distribute them efficiently over the target crop. Other adjuvants are based on crop oil. When refined or esterified, vegetable oils may be as effective as or more effective than petroleum oils or non-ionic surfactants in enhancing the phytotoxicity of herbicides. The vegetable oils can enhance the absorption, translocation and phytotoxicity of herbicides.

TABLE 1.6
LIST OF AUTHORISED ADJUVANTS

No.	Product trademark	Holder/marketing company	Chemical nature
0001	High Trees Galion	Service Chemicals Ltd.	Ethylene oxide condensate
0002	Tradename Adj.	Farm Protection Ltd.	Ethoxylated tallow amine
0003	Farmon Blue	Farm Protection Ltd.	Ethylene oxide condensate
0004	High Trees Mixture B	Service Chemicals Ltd.	Ethylene oxide condensate
0005	Cropspray 11E	Chiltern Farm Chemicals	Mineral oil
0006	Tripart Cropspray 11E	Tripart Farm Chemicals	Mineral oil
0009	High Trees Non-ionic Wetter	Service Chemicals Ltd.	Ethylene oxide condensate
0010	Quadrangle Cropspray 11E	Chiltern Farm Chemicals	Mineral oil
0011	Codacide Oil	Microcide Ltd.	Vegetable oil
0013	Actipron	BP Oil Ltd.	Mineral oil
0015	Spreadite Liquid	Dow Agriculture	Ethylene oxide condensate
0017	Exell	Truchem Ltd.	Ethoxylated tallow amine
0019	Adder	Rhone Poulenc Agr. Ltd.	Mineral oil
0020	Hyspray	Fine Agrochemicals Ltd.	Ethoxylated tallow amine
0021	Atlas Adjuvant Oil	Atlas Interlates Ltd.	Mineral oil
0020	Atlas Adze	Atlas Interlates Ltd.	Ethylene oxide condensate
0023	Atlas Adherbe	Atlas Interlates Ltd.	Mineral oil
0024	Clifton Clyphosate Additive	Clifton Farm Chem.	Ethoxylated tallow amine
0025	Planet	Industrial Detergents UK Ltd.	Alkyl polyglycol ether
0026	Genamin T-200 C ¹	Monsanto plc	Ethoxylated tallow amine
0027	Sprayprover	Fine Agrochemical Ltd.	Mineral oil
0028	Clifton Wetter	Clifton Farm Chemicals	Ethylene oxide condensate
0030	Genamin T-200 N ¹	Monsanto plc	Ethoxylated tallow amine
0031	Emerald	Intracrop Ltd.	Di-1- <i>p</i> -menthene
0033	Agral	ICI Agrochemicals	Ethylene oxide condensate
0034	Spreader	Pan Britannica Ind. Ltd.	Ethylene oxide condensate
0035	Vassgro Spreader	L.W. Vass (Agricultural) Ltd.	Ethylene oxide condensate
0037	Spraymate Bond	Newman Agrochemicals Ltd.	Synthetic latex
0038	Spraymate LI-700	Newman Agrochemicals Ltd.	Soya phospholipids
0039	Nu Film P	Intracrop Ltd.	Di-1- <i>p</i> -menthene
0042	Ethokem T25	Midkem Agrochem. Ltd.	Ethoxylated tallow amine
0044	Frigate	ISK Biotech	Ethoxylated tallow amine
0045	High Trees Wayfarer	Service Chemicals Ltd.	Ethoxylated tallow amine
0047	Citowett	BASF UK Ltd.	Ethylene oxide condensate
0049	Ethokem C12	Midkem Agrochem. Ltd.	Ethoxylated coco amine
0050	Libsorb	Atlas Interlates Ltd.	Ethoxylated coco amine
0052	Ethokem	Midkem Agrochem. Ltd.	Ethoxylated tallow amine
0053	Sprayfast	Mandops Ltd.	Di-1- <i>p</i> -menthene
0054	Quadrangle Quadfast	Mandops Ltd.	Di-1- <i>p</i> -menthene
0055	Nu Film 17	Intracrop Ltd.	Di-1- <i>p</i> -menthene
0056	Agstock Addwett	Agstock Chemicals Ltd.	Ethoxylated tallow amine
0057	Agriwett	ABM Chemicals Ltd.	Ethylene oxide condensate
0058	Agrisorb	ABM Chemicals Ltd.	Ethoxylated tallow amine
0059	Lo-Dose	ISK Biotech	Ethoxylated tallow amine

TABLE 1.6 (CONTINUED)

No.	Product trademark	Holder/marketing company	Chemical nature
0060	Enhance	Midkem Agrochemicals Ltd.	Ethylene oxide condensate
0061	Power Spray Save	Power Agrochemicals Ltd.	Ethoxylated tallow amine
0062	Spraymate Activator 90	Newman Agrochem. Ltd.	Ethylene oxide condensate
0067	Ashlade Adjuvant Oil	Ashlade Formul. Ltd.	Mineral oil
0068	Enhance Low Foam	Midkem Agrochemicals Ltd.	Ethylene oxide condensate
0069	Sterox NJ	Monsanto plc	Ethylene oxide condensate
0071	Stick It	Quadrangle Ltd.	Ethylene oxide condensate
0072	Fyzol 11E	Schering Ltd.	Mineral oil
0073	Headland Guard	WBC Technology	Synthetic latex
0074	Headland Intake	WBC Technology	Propionic acid
0075	Mangard	Mandops Ltd.	Di-1- <i>p</i> -menthene
0079	Team surfactant	Monsanto plc	Ethoxylated tallow amine
0080	Topup Surfactant	Farmers Crop Chem. Ltd.	Ethoxylated tallow amine
0081	Power Non-ionic Wetter	Power Agrochem. Ltd.	Ethylene oxide condensate
0085	Swirl	Shell Chemicals UK Ltd.	Mineral oil
0086	Concorde	ICI Agrochemicals	Mineral oil
0087	Minder	Stoller Chemical Ltd.	Vegetable oil
0094	Tonic	Brown Butlin Group	Synthetic latex
0096	Tripart Tenax	Tripart Farm Chemicals Ltd.	Synthetic latex
0097	Tripart Acer	Tripart Farm Chemicals Ltd.	Soya phospholipids
0098	Forestry Bee	Top Farm Formulations Ltd.	Ethylene oxide condensate
0099	Pro-Mix	Service Chemicals Ltd.	Ethylene oxide condensate
0100	Non-ionic 90	Top Farm Formulations Ltd.	Ethylene oxide condensate
0102	Ethywet	Top Farm Formulations Ltd.	Ethylene oxide condensate
0103	Anphix	ANP Developments Ltd.	Ethoxylated tallow amine
0106	GS 800 Adjuvant	Midkem Ltd.	Ethoxylated tallow amine
0107	Keystone	Farm Protection Ltd.	Ethylene oxide condensate
0108	Tripart Minax	Tripart Farm Chem. Ltd.	Ethylene oxide condensate
0109	Jogral	Ind. Detergents UK Ltd.	Ethoxylated tallow amines
0110	Agropel	Ind. Detergents UK Ltd.	Vegetable oil
0111	Solar	Ind. Detergents UK Ltd.	Ethylene oxide condensate
0112	Quadrangle Q 900	Crop Care Chemicals Ltd.	Ethylene oxide condensate
0113	Polycote Prime Polymer	Ciba Geigy Agrochem. Ltd.	Poly(vinyl acetate)
0114	Polycote Polymer	Ciba-Geigy Agrochem. Ltd.	Poly(vinyl acetate)
0115	Polycote Pedigree Polymer	Ciba-Geigy Agrochem. Ltd.	Poly(vinyl acetate)
0116	Rapide	Intracrop Ltd.	Propionic acid
0117	Tripart Lentus	Tripart Farm Chemicals Ltd.	Synthetic latex
0118	Clifton Alkyl 90	Clifton Chemicals Ltd.	Ethylene oxide condensate
0119	DuPont Adjuvant	DuPont UK Ltd.	Ethylene oxide condensate
0120	Farmon Wetter	Farm Protection Ltd.	Ethylene oxide condensate
0121	Barclay Dryfast XL	Barclay Chemicals Ltd.	Di-1- <i>p</i> -menthene
0122	Stefes Spread and Seal	Stefes Plant Protection	Di-1- <i>p</i> -menthene
0123	Barclay Dryfast	Barclay Chemicals Ltd.	Di-1- <i>p</i> -menthene
0124	Amos Non-ionic Wetter	Kommer Brookwick	Ethylene oxide condensate
0125	Intracrop BLA	Intracrop	Synthetic latex
0126	Barclay Actol	Barclay Chemicals Ltd.	Mineral oil

TABLE 1.6 (CONTINUE)

No.	Product trademark	Holder/marketing company	Chemical nature
0127	SM99	Newman Agrochemicals Ltd.	Mineral oil
0128	Frigate	ISK Biotech Europe Ltd.	Ethoxylated tallow amine
0129	Lo Dose	ISK Biotech Europe Ltd.	Ethoxylated tallow amine

In addition, the use of certain inorganic salts such as ammonium sulfate, ammonium paramolybdate, iron ammonium sulfate and magnesium sulfate, together with a surfactant may greatly improve herbicidal activity. The combination of various adjuvants such as oils, surfactants, and fertilizer salts can enhance the activity of some herbicides [18–22].

To conclude, it should be said that although the behaviour of specific pesticide formulations on the environment has scarcely been studied, in this book we have given an overview of the major adjuvants used. For each pesticide, specific studies will need to be undertaken for each case. Overall, we believe that information on the formulations applied is needed in order to correlate better the analytical data with the pattern of use, physicochemical properties of each pesticide and climatological conditions. Certainly, the type of formulation and the way in which materials are applied, either by manual spraying, tractor, or plane, will help to explain their fate in the environment.

1.1.5. Setting up methods for trace determination: which pesticides, where, and at what level?

Not all pesticides are likely to contaminate water or to be addressed by analytical chemists for trace-level determination in water. The occurrence of pesticides and degradation products in water in a given region of the world will first depend on whether that area has any concern regarding the compounds. The approach is not easy, and the question has to be addressed in the country of interest.

At this point we distinguish between the different reasons for setting up methods for the trace-level determination of pesticides and their degradation products in water.

There is first a regulatory aspect regarding water for human consumption, i.e., ground water or surface water after appropriate treatment. In this case, the analytical chemist will either have a list of compounds, with their corresponding quantification limits, to be determined in well-defined types of water, or will not. Regulations for drinking water are well defined in North America with given analytical procedures, whereas in Europe the regulation for each pesticide is set at $0.1 \mu\text{g/l}$, with no specific method being given. These regulations are so stringent that they cannot be applied for all the compounds. First, it would be a waste of time to look regularly for a compound which is not and never will be used in the area, because of the type of culture.

TABLE 1.7
ADJUVANTS USED ACCORDING TO WEED SCIENCE, WEED TECHNOLOGY OR WSSA
ABSTRACTS [20]

Product trademark	Chemical nature
Agral 90	Nonylphenoxypolyethoxyethanol
Agri-Dex'	Paraffin-based petroleum oil 83%; surfactant blend 17%
Agri-Oil	Polyoxyethylene esters of polyglycol, fatty acids and polyoxyalkylene esters.
Alkasurf-OP-10	Vegetable oil
ASE-108	Acrylate polymer
Assist	Paraffin-based mineral oil
Atplus-555	Polyoxyethylene alkyl ether
BAS 145-138	1-Dichloroacetyl-hexahydro-3,3, <i>a</i> -trimethyl pyrrolo-1,2- <i>c</i> pyrimidin-6-(2 <i>H</i>)-one
Bio-veg	Vegetable oil
CC-16255	Proprietary adjuvant
CD-407	Soybean oil
Citowett Plus	Octylphenoxypolyethoxyethanol
Dash (also BCH 815)	Petroleum hydrocarbons, naphthalene and oleic oil
DC-X2-5394	Organosilicone adjuvant
Enhance	Nonylphenoxypolyethoxyethanol plus tallow fatty acid amine ethoxylate
Ethokem	87% (w/v) ethoxylated (15) tallow amine
Ethomeen	Polyoxyethylene (15) tallow amine
Kinetic	Proprietary blend of polyalkyleneoxide-modified polydimethylsiloxane and non-ionic organosilicone adjuvant
Li 700	Blend of phosphatidylcholine and propionic acid
Naphthalic anhydride	1 <i>H</i> ,3 <i>H</i> -naphtho 1,8- <i>cd</i> pyran-1,3-dione
OTC	L-2-oxothiazolidine-4-carboxylic acid
Pemulens TR-1 and TR-2	Hydrophobically modified polyacrylates
Plex	Modified poly- <i>p</i> -menthene 85%; aliphatic amines 5%
Renex36	Polyoxyethylene (6) tridecyl ether
Renex 688	(Nonoxynol 8 POE) (α (<i>p</i> -nonylphenyl)- ω -hydroxypoly (oxyethylene))
Scoil	Methylated seed oil
Silwet L-77	Silicone polyalkylene oxide-modified dimethyl polysiloxane
Silwet L-7607	Polyalkylene oxide-modified dimethyl polysiloxane
Sta-Put	Polymeric adjuvant
Sun-It II	Modified vegetable oil plus surfactant
Surfactant WK (not presently commercial)	Trimethylnonylpolyethoxyethanol
Sylgaard 309	Organosilicone surfactant
Triton 11XE	Isooctylphenylpolyethoxyethanol
Triton XR	Proprietary, alcohol base
Tween 20	Oxysorbic (20 POE) polyoxyethylene sorbitan monolaurate
Tween 80	Polyoxyethylene sorbitan monooleate
X-77	Alkylarylpolyoxyethylene glycols, free fatty acids and isopropanol

TABLE 1.7 (CONTINUED)

Product trademark	Chemical nature
Crop oil concentrate	
Diammonium phosphate, diammonium sulfate	
Emulsified oil	
Isopropylamine (analytical grade)	
Aqueous solution of nitrogen (28%)	
Methylammonium	
Methylated seed oil, methylated sunflower oil	
Mono-ammonium phosphate	
Neodol linear alcohol surfactant	
Non-emulsified oil	
Non-ionic surfactant, octoxynol surfactant, oxysorbic surfactant, surfactant	
Oil adjuvant, oil-emulsified mixture	
Paraffin wax	
Petroleum oil, petroleum oil concentrate	
Sodium bisulfate	
Urea/ammonium nitrate	

Secondly, drinking water monitoring would be much too expensive and, third, some pesticides cannot be analyzed at the regulatory level because of the lack of methods. This is the case for highly water-soluble pesticides, for which there are no methods for extraction from the aquatic environment. Therefore, it is important to be able to establish the lists of pesticides to be monitored, in order of priority based on well defined criteria, and on the local pesticide usage. European countries are now establishing local priority lists containing a limited number of pesticides, which take into account their usage, leaching properties, persistence, bio-accumulation potential, toxicity, and ecotoxicity data. It is also important to revise and update local pesticide priority lists since new products are being released and may replace those currently applied. The agrochemical market is very dynamic and the regulatory agencies should adapt quickly to new realities. This is not always the case, and even in the best situation there is always a 2–3 year period of adaptation.

A second type of analytical method relates to monitoring surveys, which aim to determine the extent of the pesticide contamination or to study their environmental behaviour, i.e., their fate, transport, degradation, or ecotoxicological effects. In these surveys, the pesticides and their degradation products are selected and analytical conditions can then be optimized depending on the water type. The selection of the pesticides is also dictated by the possibility of the pesticide's entering the water directly, as a consequence of runoff water, or by volatilization for example, or indirectly via migration through the soil.

In this chapter, we first examine the most relevant physico-chemical parameters for setting up the analytical scheme, for the extraction of the pesticides and their

trace-level analysis. The second section describes their environmental relevance in the aquatic environment and the main parameters governing their possible entry and that of their degradation products into water. The dissipation of pesticides under laboratory and in field conditions, and their toxicity ranking is also given. Overall, our intention is to give a general picture of the pesticide problem in the aquatic environment, with a few case studies that, in all cases, reflect our own experience.

1.2. CHEMICAL CLASSES AND PHYSICO-CHEMICAL PROPERTIES OF PESTICIDES

As seen above, pesticides currently used over the world are numerous and have various chemical and physico-chemical properties. That is the first difficulty for their trace analysis, because environmental chemists are faced with the analysis of many compounds with various functionalities, over a wide range of polarity, solubility and acid-base properties. Survey lists can contain more than a hundred pesticides and degradation products; an example is the National Pesticides Survey list of the US EPA (see Table 1.8).

The trace analysis of pesticides in water requires a sample-pretreatment which consists of an extraction, isolation, and concentration step, before quantitative analysis and determination via chromatographic analysis. The establishment of these methods, which is described in the following chapters, requires knowledge of some basic data. Chemical functionality, water-solubility, and polarity are important parameters for guiding the extraction procedure and the selection of the chromatographic method. If the extraction procedure contains some evaporation steps, a knowledge of the volatility is also useful. In addition, as shown later, knowledge of acid-base properties can be of great help for simply adjusting the sample's pH before extraction or for performing selective extraction-analysis based on ionized and non-ionized forms.

We now discuss the various parameters which need to be considered for trace-analysis purposes. Data have been compiled in reviews, and a variety of data can be found, depending on the difficulty of measurement of some physical parameters. We also provide definitions of a few other parameters which are useful in predicting the pesticide's environmental behaviour. Today, the agronomic efficiency and human toxicity of almost all the pesticides are well known. Less is known about the prediction of their fate and behaviour in the various environmental compartments resulting from the effect of the environmental conditions. Soil retention differs from one active ingredient to another, and also from one soil to another. Degradation routes are various and some substances can only be degraded by soil organisms whereas others are mainly degraded by biotic processes. However, despite the complexity of the behaviour of the 400–600 active ingredients used in different countries, the behaviour is strongly influenced by the physico-chemical properties, solubility in water,

TABLE 1.8
PESTICIDES AND METABOLITES INCLUDED IN THE NATIONAL PESTICIDE SURVEY (USA)

α -Chlordane	Cycloate	Methiocarb
γ -Chlordane	Cymoxanil	Methomyl
α -HCH	Dalapon	Methyl paraoxon
β -HCH	DBCP	Metolachlor
γ -HCH	DCPA	Metoxuron
δ -HCH	DCPA acid metabolites	Metoxychlor
1,2-Dichloropropane	De-ethylatrazine	Metribuzin
2,4-D	Deisopropylatrazine	Metribuzin DA
2,4-DB	Deltamethrin	Metribuzin DADK
2,4,5-T	Diazinon	Mevinphos
2,4,5-TP	Dicamba	MGK 264
3,5-Dichlorobenzoic acid	Dichlorprop	Molinate
3-Hydroxycarbofuran	Dichlorvos	Monocrotophos
3-Ketocarbofuran phenol	Dicrotophos	Napropamide
4-Nitrophenol	Dieldrin	Neburon
4,4'-DDD	Dimethoate	Norflurazon
4,4'-DDE	Dinoseb	Omethoate
4,4'-DDT	Diphenamiphos sulfone	Oxamyl
5-Hydroxy dicamba	Disulfoton sulfoxide	Parathion ethyl
Acifluorfen	Disulfoton	PCP
Alachlor	Disulfoton sulfone	Pebulate
Aldicarb	Diuron	Pendimethalin
Aldicarb sulfone	EDB	Phenvalerate
Aldicarb sulfoxide	Endosulfan I	Phosphamidon
Aldrin	Endosulfan II	Picloram
Ametraton	Endosulfan sulfate	Prometon
Ametryn	Endrin	Prometryn
Aminocarb	Endrin aldehyde	Pronamide
Atrazine	EPTC	Pronamide metabolite
Atrazine dealkylated	Ethirimol	Propachlor
Barban	Ethoprop	Propanil
Baygon (Propoxur)	Etridiazole	Propazine
Bentazone	ETU	Propham
Bromacil	Fenamiphos	Propoxur
Butachlor	Fenamiphos sulfone	Simazine
Butocarboxim	Fenamiphos sulfoxide	Simetryn
Butoxycarboxim	Fenamirol	Simetryne
Butylate	Fenitrothion	Stirofos
Carbaryl	Fenpropathrin	Swep
Carbendazim	Fenuron	Tebuthiuron
Carbofilran	Fenvalerate	Terbacil
Carbofuran phenol	Fluometuron	Terbufos
Carboxin	Fluridone	Terbutryn
Chlarneh	Folpet	Tetrachlorvinphos
Chloramben	Heptachlor	<i>trans</i> -1,3-Dichloropropene
Chloridazon	Heptachlor epoxide	<i>trans</i> -Permethrin

TABLE 1.8 (CONTINUED) TRIADIMEFON

Chlorobenzilate	Hexachlorobenzene	Triadimefon
Chlorosulfuron	Hexazinone	Tricyclazole
Chlorothalonil	Isocarbamid	Trietazine
Chlorpropham	Linuron	Trifluralin
cis-1,3-Dichloropropene	Merphos	Vamidothion
cis-Permethrin	Metalaxyl	Vernolate
Cyanazine	Metamitron	Vinclozolin

polarity and volatility which are characteristic of each compound. The chemical structures and functionalities are important indicators and there is also a similarity in the behaviour of some related compounds. Therefore, it is worthwhile to have a classification of pesticides by chemical classes or in groups of substances.

1.2.1. Chemical classes

The development of pesticides within chemically related groups is explained by the fact that when one active compound was discovered, others with similar structures were investigated. Some compounds can be classified easily and unambiguously because of their characteristic chemical structures. However, a few substances do not belong to well characterized groups and are harder to classify or can have similarities to two different groups.

The main groups are listed in Table 1.9 with compounds having similar structures, according to the classification given in *The Pesticide Manual* [17] and in the *Agrochemicals Handbook* [23]. Not all the groups are equivalent in terms of number of compounds, usage, or amounts applied. Some groups can contain more than 80 different active compounds such as the organophosphorus compounds whereas some others contain very few, such as the imidazolinones. The azoles are mainly used as fungicides, whereas the organophosphorus, organochlorines and pyrethroid groups are used mainly as insecticides, acaricides or nematocides. Other groups contain mainly herbicides, except for the carbamates which are applied as herbicides or insecticides. The most widely sold herbicide families in North America are the triazines, amides and carbamates which are those commonly applied on maize, rice and soybean (atrazine, alachlor, metolachlor, EPTC, propanil and metribuzin). In Europe, one must add phenylureas and phenoxyacetic acids (isoproturon, MCPA, mecoprop). Sulfonylureas and imidazolinones are the two herbicide groups with a significant increase in use during the last 5 years.

The chemical structures of one selected compound from each group and subgroup are given in Fig. 1.2.

TABLE 1.9
MAIN PESTICIDES GROUPS AND SUB-GROUPS

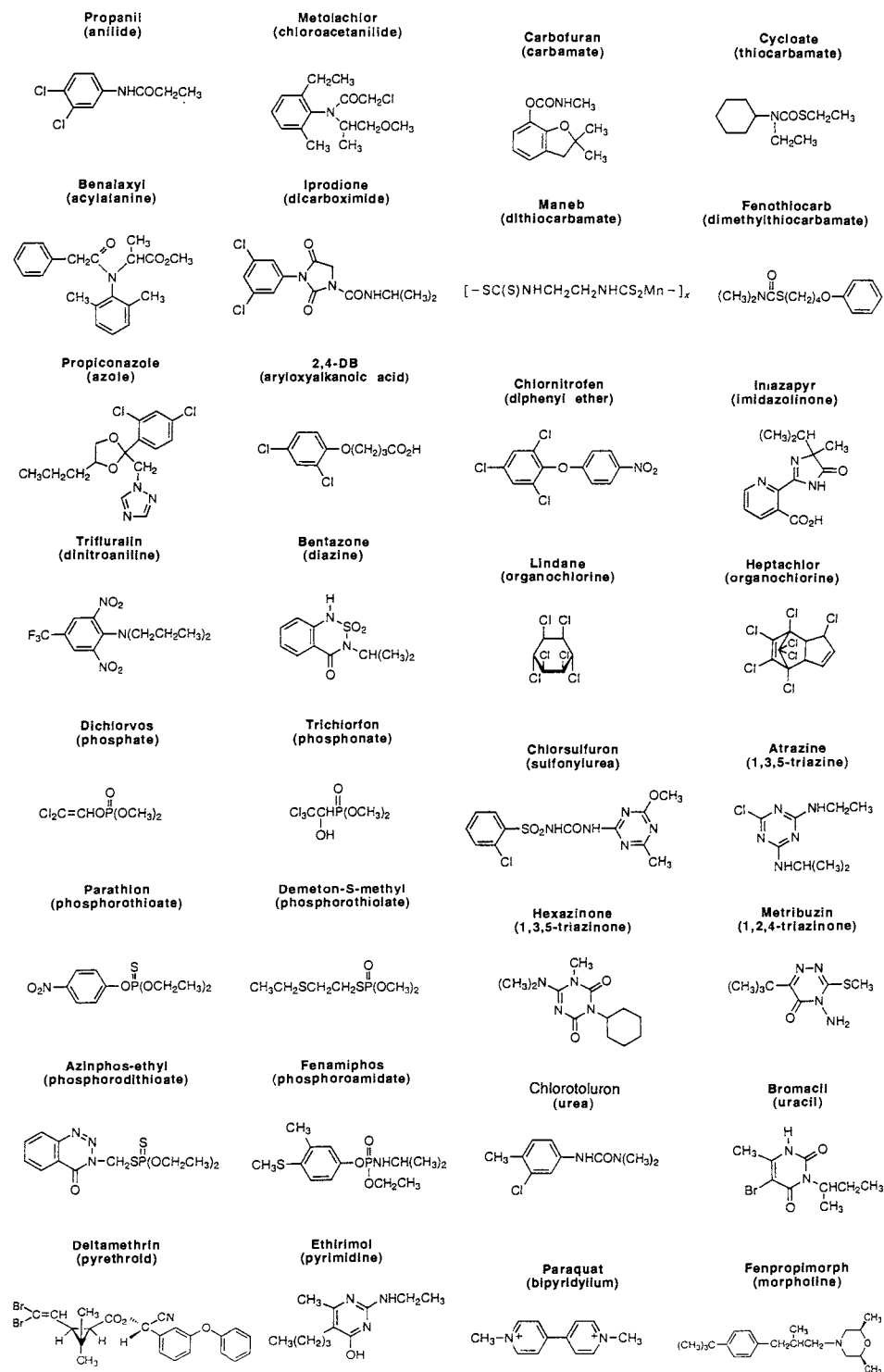
Groups	Related sub-groups
Amide	Acylanaline, chloroacetanilide, dichloroanilide
Azole	Triazole, conazole
Aryloxyalkanoic acid	Phenoxyacetic acid and salts
Dinitroaniline	
Diazine	
Carbamate	Carbamate, thiocarbamate, dithiocarbamate and dimethyldithiocarbamate
Diphenyl-ether	
Imidazolinone	
Organochlorine	
Organophosphorus	Phosphate, phosphonate, phosphorothionate, phosphorodithioate, phosphorothiolate, phosphoroamidate
Pyrethroid	
Pyrimidine	
Sulfonylurea	
Triazine	1,3,5-Triazine, 1,3,5-triazinone, 1,2,4-triazine
Urea	Phenylurea
Various	Single structure, no specific structure (benzoic acid derivatives), analyte containing too many functional groups to be easily classified

1.2.2. Physicochemical parameters: definition and data collection

The characteristic data of pesticides are usually measured according to well established protocols recognized by national and international agencies (US EPA guidelines, OECD and EU protocols, etc.). Most of the physicochemical data are measured in the laboratory under well-defined experimental conditions. Some data are characteristic of the single pesticide molecule, e.g., water solubility, vapour pressure, volatility, stability in water, photodegradation, water–octanol partition coefficient. Under given experimental conditions (temperature, pressure, pH, etc.), experiments should lead to similar values. Other, data such as the half-life in soils, or water–soil partition coefficients are measured in laboratory and/or field experiments and are strongly dependent on the experimental and environmental conditions, so they are only partly characteristic of the pesticide molecule. Therefore, data values that can be found in the literature sometimes spread over a wide range and it is not always easy to select “reliable” data.

Characteristic data have been collected for more than 250 pesticides. Our criteria were pesticides included in the various current survey lists in the US and European countries [1,3,23–25] and in recent usage. Those reported to occur in surface- or

Fig. 1.2. Chemical structure of selected compounds of the different chemical classes of pesticides.



ground water monitoring have been added. We have also included data for compounds which are difficult to analyze, such as polar compounds.

1.2.2.1. Water solubility

Water solubility is a fundamental, chemical-specific property defined as the concentration of a chemical dissolved in water when that water is both in contact and at equilibrium with the pure chemical. In Table 1.10, water solubilities are reported, measured in mg/l and at temperatures in the range 20–25°C. These values are those given in the Pesticide Manual [17] and are to be considered first for analyses of pesticides in water because they immediately indicate whether extraction from aqueous media is easy, difficult or impossible. As a general rule, a very soluble ingredient (water solubility above several g/l) cannot be extracted from water with the available extraction procedures. Very insoluble ones (water-solubility lower than 0.5–1 mg/l) are difficult to analyze at trace levels because they have a tendency to adsorb everywhere, especially on glassware; this leads to low extraction recoveries, unless some organic solvent is added to the samples prior to extraction.

Water solubility indicates the tendency of a pesticide to be removed from soil by runoff- or irrigation water and to reach the surface water. It also indicates the tendency to precipitate at the surface soil. However, this parameter alone cannot be used for predicting leaching through soil, although the distribution of pesticides in the environment is conditioned by a variety of partition coefficients into water, and several authors have shown correlations between these partition coefficients and the water solubility.

1.2.2.2. Water–octanol partition coefficient

This parameter is usually reported as a logarithm usually as $\log K_{ow}$ or $\log P_{ow}$. It is defined as the ratio of the equilibrium concentrations of the two-phase system consisting of water and *n*-octanol. The concept of K_{ow} was developed in the pharmaceutical industry as a useful index of a drug's behaviour in the body, because partitioning between water and octanol roughly mimics partitioning between water and biotic lipids. This parameter is characteristic of the lipophilicity of the molecule and gives an indication of the compound's tendency to accumulate in biological membranes and living organisms. Its determination gives data required for the registration of new organic chemicals. It is generally considered that substances with a $\log K_{ow}$ value higher than 3 can show accumulation. This risk is measured experimentally by the bioconcentration factors in aquatic organisms, and some correlations have been found between these two parameters for very hydrophobic pesticides such as the organochlorines. Some persistent organochlorines withdrawn from the market were all characterized by $\log K_{ow} > 4$.

The polarity of a molecule is strongly correlated with K_{ow} . Polarity refers to the extent to which charge is unevenly distributed within the molecule and to the occurrence of polar functional groups in it. As a rough rule, non-polar analytes are char-

TABLE. 1.10
PHYSICO-CHEMICAL PROPERTIES OF PESTICIDES^a

Pesticide	Chemical group	Usage	Solubility at 20–25°C (g/l)	Vapour pressure (Pa) at 20–25°C	Henry's Law constant (Pa m ³ mol ⁻¹)	log <i>K</i> _{ow} (a)	log <i>K</i> _{ow} (b)	p <i>K</i> _a	<i>t</i> _{1/2} (days)	<i>K</i> _{oc} (cm ³ /g)	GUS index
Acephate	Organophosphorus	I	790	2.3E-4	5.2E-8	-0.89			3	2	1.8
Acetochlor	Chloracetanilide	H	0.223	4.53E-9		3.03					
Acifluorfen	Diphenyl ether	H	0.120	1E-5		ND			14	113	
Aclonifen	Diphenyl ether	H	0.0014	1.6E-5		4.37	4.17				
Acrinathrin	Pyrethroid	I/Ac	<2E-5	3.9E-7		5.25					
Alachlor	Chloroacetanilide	H	0.242	2.9E-3	6.2E-3	ND	3.09–2.8(2)		18	120	2.4 TL
Aldicarb	Carbamate	I/Ac/N	4.93	1.3E-2	3.2E-4	ND	1.1–1.57(3)		2.4	8	1.18 IL
Aldrin	Organochlorine	I	0.000027	3.6E-2	91.2	ND	5.6–7.4(3)		365	5000	
Aldoxycarb	Carbamate	I/N/A	10	0.012		ND					
Allethrin	Pyrethroid	I	Insoluble	0.016		4.96	5.0(1)				
Ametryn	Triazine	H	0.20	3.65E-4	1.2E-4	2.63	3.07(2)	4.1	60	300	
Amitrole	Triazole	H	280	5.5E-8	1.8E-11	ND	-0.87	1.6;	0.7	127.5	×0.29 IL
Anilazine	Triazine	F	9E-6			3.02	3.12(1)	4.6	4.3	221	1.05 IL
Atrazine	Triazine	H	0.033	3.9E-5	2.9E-4	2.5	2.2–2.75(10)	1.7	50	124	3.24 PL
Azinphos-ethyl	Organophosphorus	I/AC	0.044	3.2E-4		3.18	3.4(1)		52	1465	1.43 IL
Azinphos-methyl	Organophosphorus	I	0.028	1.8E-4	3.2E-3	2.96	2.69(1)		52	1465	1.43 IL
Benalaxyl	Acylalanine	F	0.037	6.7E-4		3.4	3.4(1)				
Benazolin	Various	H	0.50	1E-7		1.34		3.04	80	22.1	5.05 PL
Bendiocarb	Carbamate	I	0.004	4.6E-3		1.7	1.64(1)	8.8	5	570	

(pH 6.5)

TABLE. 1.10 (CONTINUED)

Pesticide	Chemical group	Usage	Solubility at 20–25°C (g/l)	Vapour pressure (Pa) at 20–25°C	Henry's Law constant (Pa m ³ mol ⁻¹)	log K _{ow} (a)	log K _{ow} (b)	pK _a	t _{1/2} (days)	K _{oc} (cm ³ /g)	GUS index
Benomyl	Benzimidazole	F	0.004	<4.9E-6		ND	2.12(1)		67	1900	
Bensulfuron	Sulfonylurea	H	0.003	2.8E-12		0.62					
Bentazone	Diazine	H	0.57 (pH 7)	4.6E-4	7.4E-5	5.8 (pH 5)		3.2–3.3(2)	20	34	
Bifenox	Diphenyl ether	H	0.00035	3.2E-4	0.3	3.48			5	2414	0.43 IL
Bifenthrin	Pyrethroid	I/Ac	<1E-7	2.4E-5		>6	6.0(1)		26	240000	
Bromacil	Uracil	H	0.807 (pH 7)	4.1E-5	1.9E-3	1.87 (pH 7)	2(1)	9.27	60	32	
Bromofenoxim	Benzonitrile	H	0.0006 (pH 4)	1.3E-6	3.17 (pH 7)		5.46	134	960	2.16 TL	
Bromophos-ethyl	Organophosphorus	I	0.002	6.1E-3			5.7–6.5(2)				
Bromopropylate	Benzylate	Ac	<0.0005	1.1E-5		5.4					
Bromoxynil	Benzonitrile	H	0.130	<1E-3		ND		3.86	0.6	170	-0.39IL
Bupirimate	Pyrimidine	F	0.022	0.1E-3		3.9					
Butocarboxim	Carbamate	I	35	10.6E-3		1.1					
Butachlor	Chloroacetamide	H	0.020	0.6E-3		ND					
Butoxycarboxim	Carbamate	I/Ac	209	2.7E-4		1.1					

References pp. 89-94

Butylate	Thiocarbamate	H	0.036	1.73	0.56	1.15		13	4000	
Captan	Dicarboximide	F	0.0033	<1.3E-3	0.60	2.8	2.35-2.54(2)	1	127	0 IL
Carbaryl	Carbamate	I/PGR	0.120	4.1E-5	1.3E-4	1.59	2.29-2.36(5)	14	124	2.18 TL
Carbendazim	Benzimidazole	F	0.008	9E-5		1.5	1.40-1.52(2)	4.2	5.2	129
			(pH 7)			(pH 7)				3.24 PL
Carbetamide	Carbamate	H	3.5	<1E-4	8.8E-3	0.03		11.3	10	88.4
Carbofuran	Carbamate	I/N	0.320	3.1E-5	5.1E-4	1.52	1.3-1.63(2)		50	22
Carboxin	Phenylamide	F	0.199	2.5E-5		2.17	2.14(1)	2.8(1)	3	260
Chloramben	Benzoic acid der.	H	0.700	0.93		ND	2.8(1)			
Chlorbromuron	Urea	H	0.035	5.3E-5		ND	3.09(1)			
Chlordane (; β)	Organochlorine	I	0.0001	1.3E-3	9.02	ND	5.16-6.0(5)			
Chlorfenvinphos	Organophosphorus	I/Ac	0.145	1E-3	2.8E-4	3.85 (E)	3.1-3.8(5)			
						4.22 (Z)				
Chloridazon	Diazine	H	0.340	<1E-5	3.7	1.19	1.14-1.52(2)		31	109
Chlormephos	Organophosphorus	I	0.060	7.6		ND				
Chlormequat chlo	Quat. ammonium	PGR	>1000	<1E-5	<1.6E-9	-1.59		1.3	68	0.25 IL
Chlorobenzilate	Various	Ac	0.010	1.2E-4		ND				
Chlornitrofen	Diphenyl ether	H	0.00025	3.2E-3		5.09	3.67(1)			
Chloroneb	Various	F	0.008	0.4		ND			130	1650
Chlorothalonil	Various	F	0.0009	7.6E-5	3.4E-2	2.89			10	8551
Chlorotoluron	Urea	H	0.074	1.7E-5	5.3E-5	2.5	2.41(1)		135	175
Chlorpropham	Carbamate	H/PGR	0.089	1.3E-3	2.1E-4	ND			30	400
Chlorpyrifos	Organophosphorus	I	0.0014	2.7E-3	1.75	4.7	4.96-5.27(4)		94	4981
Chlorpyrifos-methyl	Organophosphorus	I/Ac	0.004	5.6E-3		4.2	4.3-4.32(2)			2.57 TL

TABLE. 1.10 (CONTINUED)

Pesticide	Chemical group	Usage	Solubility at 20–25°C (g/l)	Vapour pressure (Pa) at 20–25°C	Henry's Law constant (Pa m ³ mol ⁻¹)	log <i>K</i> _{ow} (a)	log <i>K</i> _{ow} (b)	p <i>K</i> _a	<i>t</i> _{1/2} (days)	<i>K</i> _{oc} (cm ³ /g)	GUS index
Chlorsulfuron	Sulfonylurea	H	27.9 (pH 7)	3E-9		-1	-0.41 (pH 2)	3.6	40	40 (pH 7)	
Chlorthal dimethyl	Benzoic acid der.	H	0.0005	2.1E-4		3.7			100	5000	
Chlorthiamid	Various	H	0.950	1.3E-4		ND					
Clofentezine	Various	Ac	2.5E-6	1.30E-7		3.1					
Clopyralid	Carboxylic acid der.	H	14 (pH 7)	1.33E-3	3.1E-5	×2.6	1.76	2.3			
Coumaphos	Organophosphorus	I	0.0015	1.3E-5		4.13					
Cyanamide	Various	H/PGR	4590	0.50		-0.82					
Cyanazine	Triazine	H	0.171	2.0E-7	3E-7	2.1	1.66-1.8(2)	1.0	19	107	2.52 TL
Cyanophos	Organophosphorus	I	0.046	0.1		2.65	2.71(1)				
Cycloate	Thiocarbamate	H	0.075	2.13E-3		3.88			30	430	
Cyhalothrin	Pyrethroid	I	4E-9	1E-6		6.8					
Cymoxanil	Acetamide	F	0.89 (pH 5)	0.8E-5	1.6E-5	4.9 (pH 7)					
Cypermethrin	Pyrethroid	I	4E-6	2.3E-7	3.9E-4	6.6	4.47(1)		30	100000	
Cyproconazole	Azole	F	0.140	3.5E-5	7.3E-5	2.9					
2,4-D	Aryloxyalkanoic acid	H	0.311 (pH 1)	1.1E-2	0.55	2.6-2.8 (pH 1)	2.8(1)	2.64	8	39 (pH 5)	1.27 TL
Dalapon	Carboxylic acid	H	Very soluble	1E-5		ND		1.8	7	0.85	3.44

References pp. 89-94

2,4-DB	Aryloxyalkanoic acid	H	0.046	Negligible	2.7E-4	ND		4.8				
DDT	Organochlorine	I	Insoluble	2.5E-5	2.36	ND	4.0-6.1(5)					
Deltamethrin	Pyrethroid	I	<2E-7	<1.33E-5	>0.5	4.6	5.4(1)					
Demeton-S-methyl	Organophosphorus	I/Ac	22	0.04	0.13	1.3						
Desmedipham	Carbamate	H	0.007	4E-8		3.4						
Desmetryn	Triazine	H	0.580	1.33E-4		2.4	2.38(1)	4.0				
Diazinon	Organophosphorus	I/Ac	0.060	1.2E-2	6.7E-2	3.3	3.11-3.81(3)		23	272		2.13 TL
Dicamba	Benzoic acid der.	H	6.500	4.5E-3	1.2E-4	3.98	2.70(1)	1.87	14	2		
						(pH 5)						
Dichlobenil	Benzonitrile	H	0.018	0.088	0.67	2.7			70	212		3.09 PI
1,3-Dichloro-propene	Organochlorine	N	2	3700	410	1.82 (E)			10	32		
						2.03 (Z)						
Dichlorprop	Aryloxyalkanoic acid	H	0.35	<1E-5	<6.E-6	1.78		3.0	10	1000		
Dichlorvos	Organophosphorus	I/Ac	8	2.1	0.19	1.9						
Diclofop	Aryloxyalkanoic acid	H	0.0008	2.5E-3		4.6			30	16000		
Dieldrin	Organochlorine		0.0002		1.12		4.3-5.4(3)12					
Diethofencarb	Carbamate	F	0.027	8.4E-3		3.02	2.89(1)					
Difenoconazole	Azole	F	0.016	3.3E-8	1.5E-6	4.2						
Diiflubenzuron	Urea	I	8E-5	1.2E-7	4.7E-4	3.89	3.69 (1)		10	10000		
Dimefuron	Urea	H	0.016	0.1E-3		2.5						
Dinoseb	Dinitrophenol	H	0.0004		51.1			4.62	50	39		4.09 PL
Dimethoate	Organophosphorus	I/Ac	0.023	1.1E-3	1.1E-4	0.70	0.50-0.79(3)	4.5	7	20		
Dinoterb	Dinitrophenol	H	0.004	2E-2	5.9E-3	ND						
Diphenamid	Acetamide	H	0.260	Negligible		ND			30	120		
Diquat	Bipyridylum	H	700	<1.3E-5		-4.6	-3.55(1)		1000	1E6		
Disulfoton	Organophosphorus	I/Ac	0.012	7.2E-3	0.22	3.95	4.02(1)		30	600		

TABLE. 1.10 (CONTINUED)

Pesticide	Chemical group	Usage	Solubility at 20–25°C (g/l)	Vapour pressure (Pa) at 20–25°C	Henry's Law constant (Pa m ³ mol ⁻¹)	log <i>K</i> _{ow} (a)	log <i>K</i> _{ow} (b)	p <i>K</i> _a	<i>t</i> _{1/2} (days)	<i>K</i> _{oc} (cm ³ /g)	GUS index
Diuron	Urea	H	0.042	1.1E–3	1.2E–3	2.85	2.68(1)		64	454	2.43 TL
DNOC	Dinitrophenol	I/Ac/ H/F	0.130	1.4E–2	0.011	ND	2.39(1)		8.5	64	2.04 TL
Endosulfan	Organochlorine	I/Ac	0.00032	8.3E–4	2.9E–2	4.74 () 4.79 (β)			50	12400	
Endothal	Carboxylic acid	H/Alg/	100	Negligible	ND			p <i>K</i> 1 3.4; p <i>K</i> 2 6.7			PGR
Endrin	Organochlorine	I	0.00023		ND		4.56–5.20(2)		4300	10000	
EPTC	Thiocarbamate	H	0.375	1E–5	1.02	3.2			45	121	3.17 TL
Esfenvalerate	Pyrethroid	I	2E–6	2E–7		6.22			35	5300	
Ethalfuralin	Dinitroaniline	H	0.0003	0.012		5.1			60	4000	
Ethiofencarb	Carbamate	I	1.8	4.5E–4		2.04					
Ethion	Organophosphorus	Ac/I	0.002	2E–4	3.2E–2	ND	5.07(1)		150	10000	
Ethirimol	Pyrimidine	F	0.253	2.67E–4		2.3					
Ethofumesate	Various	H	0.050	6.5E–4	2.2E–4	2.7			44	148	3.01 PL
Ethoprophos	Organophosphorus	N/I	0.70	0.046		3.6	2.15(1)		31	101	2.99
Etofenprox	Pyrethroid	I	E–6	3.2E–2		7.05					
Etridiazole	Azole	F	0.117	0.019		3.4	2.5(1)	2.77	103	1000	
Fenamiphos	Organophosphorus	N	0.70	0.12E–3		3.3	3.18–3.23(2)		16	267	1.89 TL
Fenamirol	Pyrimidine	F	0.014	6.5E–5	<3.3E–4	3.7			300	600	
Fenitrothion	Organophosphorus	I	0.021	1.8E–2	3.6E–3	3.43	3.30–3.60				
Fenoxaprop-P	Aryloxyalkanoic acid	H	0.0009	5.3E–7		4.6	4.28(1)		9	4490	

Fenoxycarb	Carbamate	I	0.006	8.67E-7		4.07			1	1000	
Fenpiclonil	Pyrrole	F	0.005	1.1E-9		3.86					
Fenpropathrin	Pyrethroid	Ac/I	1.4E-5	7.3E-4		6.0	3.03–5.08(2)				
Fenpropidin	Morpholine	F	0.530	1.7E-2	>5.7E-3	2.59		10.1			
Fenpropimorph	Morpholine	F	0.0043	2.3E-3	0.16	2.6	2.45(1)	6.98	67	2528	0.83 IL
Fenthion	Organophosphorus	I	0.0042	7.4E-4	2.2E-2	4.84	4.09–4.17(2)		34	1500	
Fenuron	Urea	H	0.0038	2.1E-2	2.7E-4	ND	0.96(1)				
Fenveralate	Pyrethroid	I/AC	<E-5	1.9E-5		5.01	4.42–6.2(2)		35	5300	
Fipronil	Pyrazole	I/Ac	0.002	3.70E-7		4.0					
Fluazipop	Aryloxyalkanoic acid	H	0.001	5.5E-5		4.5					
Fluometuron	Urea	H	0.110	1.25E-4		2.23	2.42(1)		85	100	
Fluridone	Pyridone	H	0.012	1.3E-5		1.87					
Fluroxypyr	Aryloxyalkanoic acid	H	0.091	3.78E-9		0.06	1.74(1)	2.94	30	34	3.65 PL
Flusilazole	Azole	F	0.054	3.9E-5	3.6E-4	3.75	3.74(1)				
Flutolanil	Phenylamide	F	0.01	1.77E-3		ND	3.70(1)				
Folpet	Various	F	Insoluble	1.3E-3	0.38	3.1			2	179	0.53 IL
Fonofos	Organophosphorus	I	0.013	2.8E-2		3.94			40	870	
Formothion	Organophosphorus	I/Ac	2.6	1.13E-4		ND					
Fosetyl (amm.)	Organophosphorus	F	120	<1.3E-5		-2.7					
Glufosinate (amm.)	Various	H	1370	<0.1E-3		<0.1			7	100	
Glyphosate	Various	H	0.012	Negligible		ND		1.0	38	167	2.81 PL
Haloxypop	Aryloxyalkanoic acid	H	0.043	<1.33E-6		1.3	3.52–4.47(2)	2.9			
Heptachlor	Organochlorine	I	6E-5	0.053	112		5.27–6.06(4)				
Hexachloro-benzene	Organochlorine		Insoluble	1.45E-3	7.12		5.44–6.18(6)				
Hexaconazole	Azole	F	0.017	1E-5	3.5E-4	3.9					
Hexazinone	Triazinone	H	0.033	8.5E-3		1.05 (pH 3)			90	54	

TABLE. 1.10 (CONTINUED)

Pesticide	Chemical group	Usage	Solubility at 20–25°C (g/l)	Vapour pressure (Pa) at 20–25°C	Henry's Law constant (Pa m ³ mol ⁻¹)	log <i>K</i> _{ow} (a)	log <i>K</i> _{ow} (b)	p <i>K</i> _a	<i>t</i> _{1/2} (days)	<i>K</i> _{oc} (cm ³ /g)	GUS index
Imazapyr	Imidazolinone	H	9.74	<1.3E-5		0.11		1.9; 3.6	90	100	
Ioxynil	Benzonitrile	H	0.050	<1E-3	<7.4E-3	ND		3.96	10	200	1.70 IL
Iprodione	Dicarboximide	F	0.013	5E-7	1.4E-5	3.0	3.1(1)		41	478	2.13 TL
Isofenphos	Organophosphorus	I	0.018	2.2E-4		4.04	4.12(1)		150	600	
Isoproturon	Urea	H	0.065	3.3E-5	9.6E-6	2.5			46	107	3.28 PL
Isoxaben	Amide	H	0.0014	5.5E-4		0.93					
Lenacil	Uracil	H	0.006	0.2E-6		2.30		10.3			
Lindane	Organochlorine	I	0.0073	5.6E-3	0.13	ND	3.66–3.85(4)		100	1100	
Linuron	Urea	H	0.081	5.1E-5	5.4E-3	3.0	2.76(1)		139	396	3.00 PL
Malathion	Organophosphorus	I/Ac	0.145	5.3E-3	2.3E-3	2.75	2.84–2.94(3)		1	1800	
Mancozeb	Dithiocarbamate	F	0.006	Negligible		ND			70	>2000	
Maneb	Dithiocarbamate	F	Insoluble	Negligible		ND			56	479	2.31 TL
MCPA	Aryloxyalkanoic acid	H	0.734	2.3E-5	4.9E-5	2.75		3.07	15	50	2.71 TL
MCPB	Aryloxyalkanoic acid	H	0.044	5.77E-5		2.79		4.84	14	20	
Mecoprop	Aryloxyalkanoic acid	H	0.734	0.31E-3	1.1E-4	1.26		3.78	2.8	127	2.74 TL
						(pH 7)					
Metam-sodium	Various	F/N/ H/I	722	Non-volatile		<1			<0.1	387	
Metamitron	Triazinone	H	1.7	8.6E-7	9.6E-9	0.83					

Metazachlor	Chloroacetanilide	H	0.43	4.9E-5	7.7E-4	2.14				
Methabenz- thiazuron	Urea	H	0.059	5.9E-6	<5.1E-3	2.64		135	527	2.72 TL
Methamidophos	Organophosphorus	I/Ac	>200	2.3E-3		×0.8		2.6	1.7	1.56 IL
Methidathion	Organophosphorus	I/Ac	0.20	2.5E-4		2.2	2.42-2.57(2)	4.5	163	1.17 IL
Methiocarb	Carbamate	Mol/I/ Ac/BR	0.027	1.5E-5		3.34	2.92(1)	41	564	2.01 TL
Methomyl	Carbamate	I/Ac	57.9	6.65E-3	6.5E-5	1.24	0.08-0.13(2)	30	72	
Methoxychlor	Various	I	0.0001	Very low		ND	3.31	120	80000	
Metobromuron	Urea	H	0.330	4E-4	3.1E-4	2.41	2.38(1)			
Metolachlor	Chloroacetanilide	H	0.488	4.2E-3	9E-4	2.9	3.13-3.28(2)	101	175	3.52 PL
Metoxuron	Urea	H	0.678	4.3E-3	1.5E-3	1.6	1.64(1)			
Metribuzin	Triazinone	H	1.050	5.8E-5	<2.4E-4	1.58	1.70(1)			
Metsulfuron	Sulfonylurea	H	2.79	3.3E-10		-1.7		3.3	30	35
			(pH 7)							
Mevinphos	Organophosphorus	I/Ac	Miscible	1.7E-2		0.13		3	44	
Molinate	Thiocarbamate	H	0.088	0.746		2.88	3.21(1)	21	190	
Monocrotophos	Organophosphorus	I/Ac	Miscible	2.9E-4		-0.22		30	1	
Monolinuron	Urea	H	0.735	1.3E-3		2.2	2.30(1)			
Monuron	Urea	H			3.0E-3		1.98(1)			
Myclobutanil	Azole	F	0.142	2.13E-4		2.95				
Naled	Organophosphorus	I/Ac	Insoluble	0.27		ND	1.38(1)	1	180	
Napropamide	Amide	H	0.073	5.3E-4		3.3		70	700	
Neburon	Urea	H	0.005	ND		ND				
Norflurazon	Pyridazinone	H	0.028	2.8E-6		2.45	2.30(1)	30	700	
Omethoate	Organophosphorus	I/Ac	Soluble	3.3E-3		-1.1				
Oxamyl	Carbamate	I/Ac/N	280	3.1E-2	2.6E-4	-0.4	-0.47(1)	4	25	
Oxydemeton- methyl	Organophosphorus	I	Miscible	3.8E-3		-0.7	1.38(1)	3.4	75	1.13 IL

TABLE 1.10 (CONTINUED)

Pesticide	Chemical group	Usage	Solubility at 20–25°C (g/l)	Vapour pressure (Pa) at 20–25°C	Henry's Law constant (Pa m ³ mol ⁻¹)	log K _{ow} (a)	log K _{ow} (b)	pK _a	t _{1/2} (days)	K _{oc} (cm ³ /g)	GUS index
Paraquat	Bipyridylum	H	700	<0.1E–3		ND	–5.0		1000	<1E6	
Parathion	Organophosphorus	I/Ac	0.011	8.9E–4	1.23E–2	3.83	2.15–3.93(3)		14	5000	
Parathion-methyl	Organophosphorus	I	0.055	0.2E–3	2.1E–2	3.0	1.8–3.04(5)		18.5	236	2.06 TL
Pebulate	Thiocarbamate	H	0.060	4.7	11.7	3.83			14	1430	
Penconazole	Azole	F	0.073	2.1E–4		3.72					
Pendimethalin	Dinitroaniline	H	0.0003	4.0E–3	3.78	5.18					
Pentachloro-phenol		I/F/H	0.080	16	4.4E–2		3.6–5.0(3)	4.71			
Permethrin	Pyrethroid	I	0.0002	4.5E–5		6.1	5.8–6.6		30	100000	
Phenmedipham	Carbamate	H	0.005	1.33E–9	8E–8	3.59					
Phenthoate	Organophosphorus	I/Ac	0.011	5.3E–3		3.7	2.89–3.96(2)				
Phorate	Organophosphorus	I/Ac/N	0.050	8.5E–2	0.65	3.9	3.83–4.26(2)		60	1000	
Phosalone	Organophosphorus	I/Ac	0.0017	<6.7E–5		3.3	4.3–4.38(2)		21	18000	
Phosmet	Organophosphorus	I/Ac	0.025	6.5E–5	9.5E–4	2.95					
Phosphamidon	Organophosphorus	I/Ac	Miscible	2.2E–3	0.36	0.79					
Phoxim	Organophosphorus	I	0.0015	2.1E–3		3.4	4.39(1)				
Picloram	Carboxylic acid	H	0.430	8.2E–5	3.4E–5	ND	0.30	2.3	90	16	
Pirimicarb	Carbamate	I	3	0.97E–3		1.7			108	741	2.30 TL
Pirimiphos-ethyl	Organophosphorus	I	0.0023	0.68E–3		5.0	4.85(1)				

Pirimiphos-methyl	Organophosphorus	I/Ac	0.0099	2E-3		4.2	4.2(1)		10	1000	
Prochloraz	Azole	F	0.0344	1.50E-4	1.7E-3	4.4		3.8	171	7310	0/30 IL
Procymidone	Carboximide	F	0.0045	1.8E-2	1.1	3.14					
Prometon	Triazine	H	0.750	0.306E-3	9E-5	ND	2.99-3.1(2)	4.3	500	1500	
Prometryn	Triazine	H	0.033	0.169E-3	5E-4	3.1	3.34-3.48	4.1	41	258	2.56 TL
Propachlor	Chloroacetanilide	H	0.613	30.6E-3	1.1E-2	1.62-2.3	1.41(1)		5	68	1.52 IL
Propanil	Anilide	H	0.130	2.6E-5	3.6E-3	3.3	2.8(1)		1	149	
Propazine	Triazine	H	0.005	3.9E-6	1.0E-4	ND	2.91-3.02(2)	1.7	135	154	
Propham	Carbamate	H/PGR	0.250	Considerable		ND	2.6(1)		7	18.7	2.31 TL
Propiconazole	Azole	F	0.100	5.6E-5	4.1E-4	3.72	3.65(1)		96	906	2.07 TL
Propoxur	Carbamate	I	1.90	1.3E-3	0.13	1.56	1.55-1.58(2)		33	30	
Propyzamide	Amide	H	0.015	5.8E-5	0.19	3.1-3.3			25	253	2.23 TL
Prosulfocarb	Thiocarbamate	H	0.0132	6.9E-5		4.65					
Pyrazophos	Organophosphorus	F	0.0042	0.22E-3		3.8					
Pyridate	Various	H	0.0015	1.30E-7	3.3E-5	>3					
Quinmerac	Carboxylic acid	H	0.223	<1E-5		×1.11		4.3			
Quizalofop	Various	H	0.0003	0.86E-9		4.3					
Rimsulfuron	Sulfonylurea	H	<0.01	1.5E-6		0.3		4.0			
						(pH 7)					
Rotenone	Various	I/Ac	0.015	<1E-3		ND					
Simazine	Triazine	H	0.0062	2.94E-6	3.4E-4	2.1	1.51-2.26(5)	1.7	59	115	3.43 PL
Simetryn	Triazine	H	0.450	ND		ND	2.66-2.8(2)				
Sulfometuron	Sulfonylurea	H	0.244	7.3E-11		1.17		5.2	20	78 (pH 7)	
			(pH 7)			(pH 5)					
Sulfotep	Organophosphorus	I/Ac	0.010	1.4E-2	1	4.0					
Tebuconazole	Azole	F	0.032	1.3E-6	9.3E-6	3.7					
2,4,5-T	Aryloxyalkanoic acid	H	0.005	8E-7			0.6-0.8(2)				

TABLE 1.10 (CONTINUED)

Pesticide	Chemical group	Usage	Solubility at 20–25°C (g/l)	Vapour pressure (Pa) at 20–25°C	Henry's Law constant (Pa m ³ mol ⁻¹)	log K _{ow} (a)	log K _{ow} (b)	pK _a	t _{1/2} (days)	K _{oc} (cm ³ /g)	GUS index
Terbacil	Uracil	H	0.710	6.25E-5	1.8E-5	1.9		9	120	55	
Tebuthiuron	Urea	H	2.50	0.27E-3		1.8					
Teflutrln	Pyrethroid	I	2E-5	8E-3		6.5					
Temephos	Organophosphorus	I	3E-5	ND	5.8E-3	4.9	3.9(1)				
Terbufos	Organophosphorus	I/N	0.0045	3.46E-2	1	4.5	4.48(1)		5	500	
Terbumeton	Triazine	H	0.130	0.27E-3		3.04	3.1(1)	4.6			
Terbutylazine	Triazine	H	0.0085	0.15E-3		3.04	3.02–3.74(2)	2.0	1145	306	3.11 PL
Terbutryn	Triazine	H	0.022	0.225E-3	1.3E-3	3.65	3.53(1)	4.3	66	657	2.15 TL
Tetrachlorvinphos	Organophosphorus	I/AC	0.011	5.6E-6		ND					
Tetraconazole	Azole	F	0.150	1.6E-3		3.53					
Tetramethrin	Pyrethroid	I	0.0046	9.44E-4		4.5	4.7(1)				
Thiabendazole	Benzimidazole	F	<0.05 (pH 5–12)	Negligible		ND		4.7	403	2500	
Thifensulfuron	Sulfonylurea	H	0.230	1.7E-8		0.2 (pH 6)		4.0	12	45 (pH 7)	
Thiobencarb	Thiocarbamate	H	0.030	2.2		3.4	3.40–3.42(2)		21	900	
Thiodicarb	Carbamate	I/Mol	0.035	5.7E-3		ND			7	350	
Thiofanox	Carbamate	I/A1c	5.2	2.26E-2		ND	1.65(1)				
Thiometon	Organophosphorus	I/Ac	0.200	2.3E-2		3.5					

Thiram	Thiocarbamate	F	0.018	2.3E–3		1.73		23	3.4	4.72 PL
Tralomethrin	Pyrethroid	I	0.070	1.7E–11		5.0		27	100000	
Triadimefon	Azole	F	0.064	2E–5		3.11		40	340	2.35 TL
Triadimenol	Azole	F	0.062	<1E–3	4.2E–3	3.1–3.3	2.9–3.1(2)	114	228	3.38 PL
Tri-allate	Thiocarbamate	H	0.004	1.6E–2	1.02	ND	4.66(1)	103	1857	1.47 IL
Triasulfuron	Sulfonylurea	H	0.815	<2E–6		–0.6		4.6		
			(pH 7)			(pH 7)				
Triazophos	Organophosphorus	I/Ac/N	0.030	0.39E–3		3.3	3.55(1)			
Trichlorfon	Organophosphorus	I	120	2.1E–4	1.7E–6	0.43	0.43(1)	29	29	3.71 PL
Triclopyr	Aryloxyalkanoic acid	H	8 (pH 7)	2E–4		–0.45		3.97		
						(pH 7)				
Tricyclazole	Azole	F	1.6	2.7E–5		1.4				
Tridemorph	Morpholine	F	0.012	6.4E–3	0.12	4.2		7.5	33	2034
						(pH 7)				0.79 IL
Trietazine	Triazine	H	0.020	ND		ND	2.9–3.0(2)			
Trifluralin	Dinitroaniline	H	0.0002	9.5E–3	4.02	5.2	3.97–5.1(2)	170	6417	0.43 IL
			(pH 7)			(pH 8)				
Vamidothion	Organophosphorus	I/Ac	Very soluble	Negligible		ND	1.08(1)			
Vernolate	Thiocarbamate	H	0.090	1.39	2.05	3.84		12	260	
Vinclozolin	Dicarboximide	F	0.0034	1.6E–5	1.3E–3	3.0	2.9–3.0(2)	30	267	2.32
Warfarin	Coumarin	Ro	0.017	9		ND	0.05–2.72(2)			
Zineb	Thiocarbamate	F	0.010	<1E–5		<1.3				
Ziram	Thiocarbamate	F	0.065	<1E–6		1.08		40	10.4	

^alog K_{ow} (a), from *Pesticide Manual*; log K_{ow} (b), from Refs. [29–31]. TL, transient leacher; IL, improbable leacher; PL, potential leacher.

acterized by log K_{ow} values above 4–5, whereas polar analytes have log K_{ow} values below 1 or 1.5. Between these two values, compounds are classified as moderately polar.

Among the various modes of liquid chromatography the most common is reversed-phase chromatography. This involves hydrophobic interactions in the retention mechanism, so K_{ow} is applied for choosing liquid-chromatography conditions for the analysis of pesticides. A correlation exists between the retention factors of the analytes and their log K_{ow} values. The same interactions exist when reversed-phase sorbents such as *n*-octadecyl silicas are used for extraction of pesticides in water, and log K_{ow} is one of the parameters needed for choosing suitable conditions for the extraction.

In addition to permitting the prediction of bioaccumulation in aquatic and terrestrial organisms, K_{ow} has proved valuable for prediction of mobility and persistence in soils, and of soil sorption [26–28]. Hydrophobic interactions also occur in the sorption of pesticides in soils containing large amounts of organic matter and so it is not easy to explain why some correlations were found between soil–water partition coefficients and log K_{ow} . However, K_{ow} alone cannot be considered to be an indicator of soil affinity because it merely represents a partition between two well-defined non-miscible phases, whereas the real soil sorption process involves other mechanisms such as partition, adsorption, ion-exchange, complexation and precipitation.

The value of this parameter for predicting analytical data and environmental behaviour has led to extensive compilations of K_{ow} values. Although required for registration, the K_{ow} values of pesticides have not been provided by manufacturers prior to publication of the last edition of the *Pesticide Manual*. Therefore, in Table 1.10 we have reported log K_{ow} values in two columns, one with the single value reported in the *Pesticide Manual* (a) and the second one with a range of values, as given in the recent compilations (b) [29–31]. Because the definition of K_{ow} is unambiguous, a single data value should be expected from laboratory measurements. In fact, uncertainty is frequently reported in K_{ow} measurements, and is explained by the difficulties and the various means of determination [29].

A number of methods have been developed for the experimental measurement and for the estimation of partition coefficients. A direct experiment uses the classical “flask-shaking”, and is endorsed by the Organization for Economic Co-operation and Development (OECD) Council and widely used as a recognized test method [32]. The chemical is mixed with an appropriate water–octanol mixture and shaken until it reaches equilibrium. This method only applies to pure substances, is time-consuming, and is hardly applicable to surface-active substances and hydrophobic compounds, because of the very low concentrations to be measured in the aqueous phase. Therefore, other ways of determination have been developed and received attention such as the “generator column” or “slow stirring” methods [33,34]. However, it is clear that direct experimental measurement of K_{ow} is difficult and is not applicable to many compounds of environmental significance.

Values of K_{ow} can also be estimated by indirect measurements, using reversed-

phase liquid chromatography. This method was endorsed by the OECD Council in 1989 [35]. A set of reference compounds needs to be run for the determination of an unknown value, to produce calibration curves of $\log K_{ow}$ against chromatographic measurements of retention factors. This method is fast and has been shown to be applicable for a range of $\log K_{ow}$ between 0 and 6. However, this method is dependent on the range of mobile phase used for measuring the retention factor (see Chapter 4) and on the selected reference compounds. The use of chemicals whose chemical structures do not differ too significantly is recommended [31].

Values of K_{ow} can be estimated using calculations based on substituent constants defined, in a manner analogous to the Hammett constants, by Hansch and Leo [36,37] and hydrophobic fragmental values by Rekker [38]. In this method, compounds are logically divided into fragments and $\log K_{ow}$ values are calculated by the addition of fragment values. However, these calculations are hard to apply to molecules having complex structures, because mutual interactions between the various substituents have to be taken into account.

As a consequence of the variety of methods used for the measurement or estimation of K_{ow} , the variability in reported values can be very large, up to 200-fold for some compounds. This is seen from Table 1.10, where both the range and the number of values is reported, mainly from the review of Noble [31]. The *Pesticide Manual* gives the single value, provided by the manufacturers, with no indication of the way in which it was obtained. Therefore, it is of interest to add the range in that table, so the true value will be within this range of variation.

Some authors have reported that correlations with the solubility of compounds allow it to be a good predictor of partition coefficients [39,40]. However, this relationship is theoretically possible only for homologous series or for structurally related compounds. In Ref. [40], the authors have examined the relationship for 61 organophosphorus and carbamate insecticides and have found a regression for $\log K_{ow}$ versus $\log S$ (corrected for melting points, $n = 58$, $r = 0.975$). However, they clearly concluded that the regression equation could only serve as a cross-check for determining K_{ow} , but not for accurate prediction. An example of the relationship is shown in Fig 1.3 for a set of selected K_{ow} values (mostly experimental values) of pesticides belonging to various chemical groups, using mass and molar solubilities. Since it is a log-log relationship, it is evident that the range of $\log K_{ow}$ values is 2–5 for a solubility in the range 0.1–1 mg/l [29]. The authors have tried to include other parameters, using multiple regression and principal component analysis, and have shown that K_{ow} correlates better with the combination of solubilities than with either the melting point or the connectivity index. Many studies are now being made for the calculation of $\log K_{ow}$ using neural networks.

However, some special attention should be given to the partitioning of non-polar pesticides having low solubilities. This is because they have a high potential to adsorb everywhere, as on soil or aqueous particulate matter in the environment or on laboratory equipment in experiments.

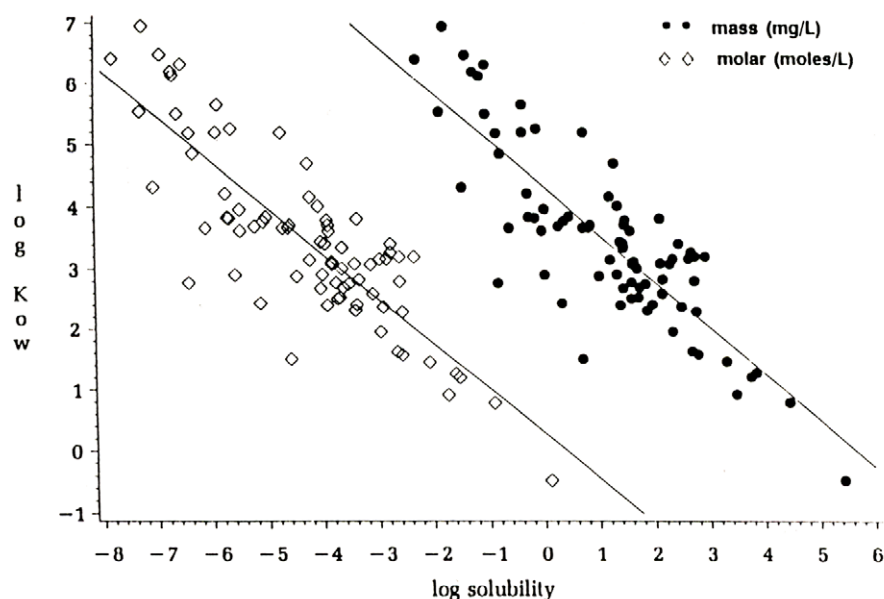


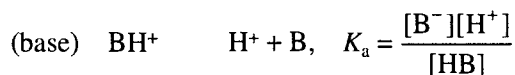
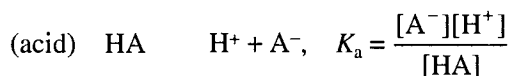
Fig. 1.3. Comparison between mass- and molar-solubility measurements as predictors of partition coefficients for various pesticides. From Ref. [18] with permission.

1.2.2.3. Acid-base ionization constants

Ionic pesticides behave differently from non-ionic pesticides. It is therefore important to know which pesticides are capable of ionization within the normal soil/water environmental pH range of 5–8. Since soils have a tendency to be negatively charged, anionic compounds may be potential leachers whereas cationic compounds will be strongly retained.

This knowledge is also important for performing trace analysis and especially for extractions from water, because it is much easier to extract a non-ionic compound than an ionic one. A simple pH adjustment of the sample can help greatly in extraction recovery, which requires a knowledge of the ionization constant values. The water solubility of ionic species is always much higher than that of their non-ionic form. Note that especially at a pH around the pK_a value(s) the solubility, mobility and volatility will be pH dependent.

The acid ionization constant, K_a , is related to the equilibrium concentration of the non-ionic and ionized forms by



The ionization constant is usually expressed as pK_a ($= -\log K_a$). For bases, old ionization constants, denoted pK_b values, can still be found in the literature (representative of $B + H_2O \rightleftharpoons BH^+ + OH^-$). They are simply related to pK_a values by the relationship $pK_a = 14 - pK_b$.

The higher the pK_a value, the weaker is the acid and its tendency to be ionized. When the water or soil pH is around its pK_a , 50% of the species are ionized. An acid is essentially all in its ionized form at pH values two units above the pK_a value and a basic compound is essentially totally protonated at pH values two units below the pK_a . Therefore, acidic pesticides with pK_a values below 3–4 will be mobile whereas basic pesticides with pK_a values higher than 10 will be the most retained by soils.

Experimental measurements of pK_a are easily made and available values are collected in Table 1.10, mainly from the *Pesticide Manual*. Phenoxyalkanoic acids, sulfonylureas and other herbicides such as benazolin, bromoxynil, dicamba, ioxynil, and fluroxypyr have pK_a values around 3–4.

The acid-base properties of the degradation products can be very different from the parent compounds. As an example, the pK_a value of atrazine is 1.6, so this compound is never ionized in the environment, whereas the pK_a value of the hydrolyzed transformation product, hydroxyatrazine is around 4.5. However, the pK_a values of degradation products are seldom available.

1.2.2.4. Vapour pressure

The vapour pressure is another chemical-specific property, defined as the partial pressure of a chemical, in the gas phase, in equilibrium with the pure solid or liquid chemical. Vapour pressures are very temperature-dependent. This parameter governs the distribution between liquid and gas phase or between solid and gas phase. It is expressed in Pa and, for our purposes, measured at temperatures in the range 20–25°C. However, many values are reported in mmHg. Experimental measurements are not easily made especially because many plant-protection products have low vapour pressure values. They are not always measured at ambient temperature, but can be extrapolated from measurements at higher temperatures using the Clapeyron equation. Reported vapour pressure values often exhibit wide discrepancies, up to tenfold between different authors. The values reported in Table 1.10 are extracted from the *Pesticide Manual* [17].

1.2.2.5. Henry's Law constant

Henry's Law constant, denoted H or K_H is a partition coefficient defined as the ratio of a chemical's concentration in air to its concentration in water at equilibrium. This parameter is important in several respects. The tendency of pesticides to volatilize from water solution into air is largely determined by their H values, a high value favouring volatilization. Air–water partitioning is important in studies of pesticide associations with rain, cloud water, fog water, or in the alveoli of human and other animals' lungs [41]. Samples containing such compounds must be handled

carefully in order to avoid loss, and evaporation steps should not be included in the sampling process. Pesticides having large H values may be analyzed by headspace analysis or gas-stripping.

The H values of compounds are more appropriate indicators of their volatilization than the single value of the vapour pressure because they represent partitioning coefficients. Even if, at a first approximation, vapour pressure values are useful for indicating and classifying compounds by groups of increasing volatility, a weak vapour pressure does not always indicate a negligible volatilization. As an example, DDT has a weak vapour pressure, but a low water solubility, so its volatilization cannot be totally negligible.

Suntio et al. have presented a compilation and critical review of Henry's Law constants for many pesticides [41]. The value of H can be expressed in either a dimensionless form or with units. In the dimensionless form, the same units of concentration are used in both the air and the water phases. The dimensionless form can be converted into the dimensional form by multiplying by RT , thus converting the air concentration to units of pressure, with the use of Ideal Gas Law. By use of Henry's law, H can be conveniently calculated as the ratio of the liquid or solid vapour pressure and solubility. Therefore, H is often reported in $\text{Pa m}^3 \text{mol}^{-1}$, with the vapour pressure in Pa, and the solubility of the chemical in water expressed as a molar fraction in mol m^{-3} .

The H values can be estimated from experimentally-determined solubilities and vapour pressures. The preferred methods involve the flow of air or water through "generator columns" [42]. Gas chromatographic methods can also be used for determining vapour pressures [43]. An "equilibrium partitioning in closed system" method was shown to be suitable for compounds having high H values ($>100 \text{ Pa m}^3 \text{mol}^{-1}$) [44]. Other methods involve a flow system in which the concentration of the chemical in water with a steady stream of gas is measured as a function of time [45]. As a consequence of these variations in methods, the H values reported by different authors exhibit wide discrepancies, as is found for values of vapour pressure.

The values reported in Table 1.10 come mainly from the critical review of Suntio et al., who calculated H values from selected vapour pressure and solubility values [41].

It is generally considered that compounds with H values $< 10^{-5} \text{ Pa m}^3 \text{mol}^{-1}$ have little tendency to volatilize.

1.2.2.6. Normalized soil sorption coefficient (K_{oc})

One of the most critical factors for assessing the potential mobility of most pesticides in the soil compartment is the distribution between the solid and liquid phases of soil. This partitioning presents a difficult problem since the types of soil in the environment vary enormously. A first characterization is the measurement of the simple "sorption" coefficient, K_d , defined as the ratio of the concentration of the chemical adsorbed on soil to the concentration of pesticide in the soil solution. This can be measured simply by experiments endorsed by the OECD Council in 1981

[46]. Various studies have demonstrated that for adsorption K_d values measured in a range of soils, good correlations were obtained between K_d and the organic matter content of the soil. This indicates that the principal adsorption mechanism involves an interaction between the pesticide and the organic matter component of the soil. Therefore, the adsorption coefficient is normalized to take into account the different soil organic matter or organic carbon content, and K_d values are expressed per unit of organic matter as K_{om} , or per unit of organic carbon as K_{oc} .

$$K_{om} = 100K_d/(\% \text{ organic matter})$$

$$K_{oc} = 100K_d/(\% \text{ organic carbon})$$

The K_{oc} values are more commonly reported in the literature than K_{om} values. They are referred to as “soil organic carbon sorption coefficients” and expressed in $\text{cm}^3 \text{ g}^{-1}$. The environmental relevance of this parameter is important for leaching properties in ground water and is discussed later in this chapter.

A range of K_{oc} values can be found in the literature for the same pesticide because of sensitivity to the characteristics of the soils used for measurements and to the experimental and environmental conditions. Most of the K_{oc} values reported in Table 1.10 were selected by Wauchope et al. [30]. Pesticides with K_{oc} values below 50 are considered to be highly mobile; values of 150–500 signify moderately mobile, and above 2000, slightly mobile compounds.

1.2.2.7. Field half-life

The degradation of pesticides in the sub-surface of soils is often described using a modified first-order equation:

$$C_t = C_0 \exp[-k(t - t_0)]$$

where C_t and C_0 are the concentrations at times t and 0 (units typically in days), and k is a time constant expressed in the same reciprocal units. The “half-life”, $T_{0.5}$ is defined as the time required for the pesticide to undergo dissipation or degradation to half of its initial concentration. If the above equation is appropriate, the half-life is independent of the initial time and concentration. However, there is not a single half-life for pesticides, and measurements depend strongly on the environmental conditions (soil, site, climate, soil microbial activity, etc.). Both laboratory and field measurements are reported. Laboratory measurements have the advantage of being performed under well-controlled conditions of temperature, light and humidity, but are far from the “real” conditions, because of the lack of vegetation and intense microbial activity. Field experiments are certainly more reliable because they are made under real usage conditions. These include all the dissipation processes, i.e., biotic and abiotic degradation, migration by volatilization, runoff, leaching, plant

absorption, etc. As a consequence, the exponential decay function can only be an approximation. Later in this chapter, we give a few examples of field half-lives. This is a subject that needs further research, since half-life data reported in the literature often do not reflect the local climatological conditions. Probably the tropical countries need particular attention in this respect. Many of these countries are still under development and use large amounts of pesticides for cultivation of crops such as bananas, rice and coffee. Since most of the half-life studies have been reported for the US or Europe, they are not applicable to tropical countries, owing to the very different climatological conditions, and soil–water composition.

Wauchope et al. [30] noted that data collected from the temperate climate zone can vary over a factor of three.

For the reasons given above, $T_{0.5}$ values are certainly the most uncertain. The values reported in Table 1.10 are mainly field data selected by Wauchope et al. [30].

1.2.2.8. Mobility index

Gustafson considered 22 pesticides for which some physico-chemical and environmental data were available and proposed the Ground Ubiquity Score (GUS index) which produces a leaching classification based on soil half-life and K_{oc} :

$$\text{GUS} = \log T_{0.5} \times (4 - \log K_{oc})$$

These values are presented in Table 1.10, calculated from $T_{0.5}$ and K_{oc} values [1]. This parameter is discussed later.

1.3. ENVIRONMENTAL RELEVANCE IN THE AQUATIC ENVIRONMENT

In evaluating the potential impact of a pesticide, one must consider its distribution and fate in the soil and in water. One should also consider the patterns of its use in localized areas, such as greenhouses or around buildings, with a limited environmental contamination, or whether it is widespread, as with the normal field-use of pesticides, or distributed by helicopter or plane. An example of the different types of pesticide pollution is shown in Fig. 1.4.

Figure 1.4 shows that the important causes of pollution are: (i) direct pollution of wells and well borings by surface runoff waters, spills, and back-siphoning of dilute pesticide solution from spray equipment to the well; (ii) leaching from sites where tractors and sprayers are rinsed; (iii) leachate from municipal landfills and small waste disposals; (iv) infiltration of pesticide polluted water from lakes and rivers; and (v) leaching of surface-applied pesticides through mass flow or preferential-flow macropores [47].

It is essential to consider the environmental distribution of pesticides and their transformation products. Most pesticides are applied directly to the soil, or will reach

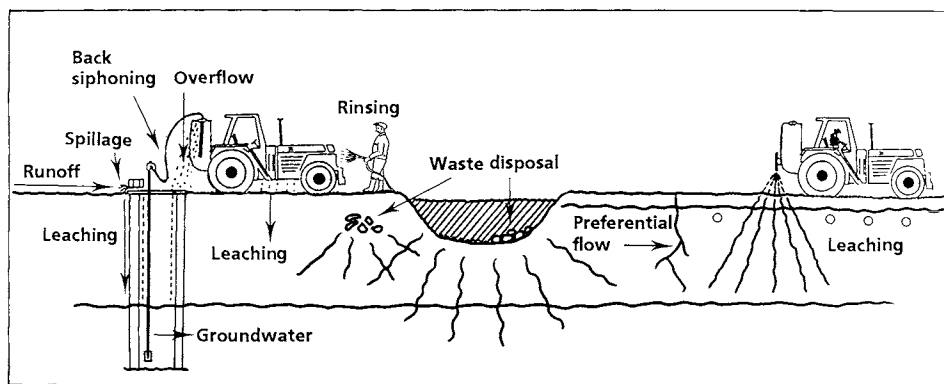


Fig. 1.4. Pesticide pollution of water may be caused either by direct pollution of wells or well-boring, by leaching from point sources on soil surface, or in waste disposal sites or by leaching after treatment.

the soil in spray after-drift or runoff during the spraying of crops. Studies on the fate in soil are therefore essential. Also, where the pesticide is applied directly to the water, via leaching, runoff or in the ways shown in Fig. 1.4, significant amounts of chemicals or their degradation products will reach the aquatic systems. In this respect, studies on the fate of pesticides in soil/water or sediment/water are being undertaken, and will provide knowledge on this behaviour. Such studies should supply information on: (i) rates of degradation of the pesticide in the soil; (ii) the nature and likely persistence of the major transformation products; (iii) the distribution of the pesticide and its transformation products via leaching, runoff and volatilization; (iv) possible contamination of aquatic systems; and (v) the fate of the pesticide and/or its transformation products in the aquatic system (when needed). Such information is vital for the prediction of the environmental fate of a pesticide.

A study of the Minnesota river watershed, where atrazine, alachlor and cyanazine are being applied, showed that the intensive cultivation practices and subsequent soil erosion, resulted in high suspended sediment concentrations of 100–1000 mg/l in the river. In addition, because the soil porosity is poor, much of the farmland had been tilled to enhance drainage. Figure 1.5 describes the dominant flow paths for drainage from agricultural fields in the Minnesota river watershed. The overall delivery of the water to the river is dramatically enhanced by subsurface drainage networks which increase the drainage by facilitating the removal of infiltrating water or collecting pond water and removing it through vertical tiles. Water removed to the tile networks is ultimately routed to drainage ditches, tributaries or naturally draining topography. Overland flow and removal of pond water via vertical tile drains are considered here as surface transport pathways (pathway A). Subsurface flow (pathway B) corresponds to water that travels through the unsaturated zone and is collected and transported by the tile lines, as well as water that infiltrates to the water table (pathway C) and then moves to the river in the local and /or regional groundwater

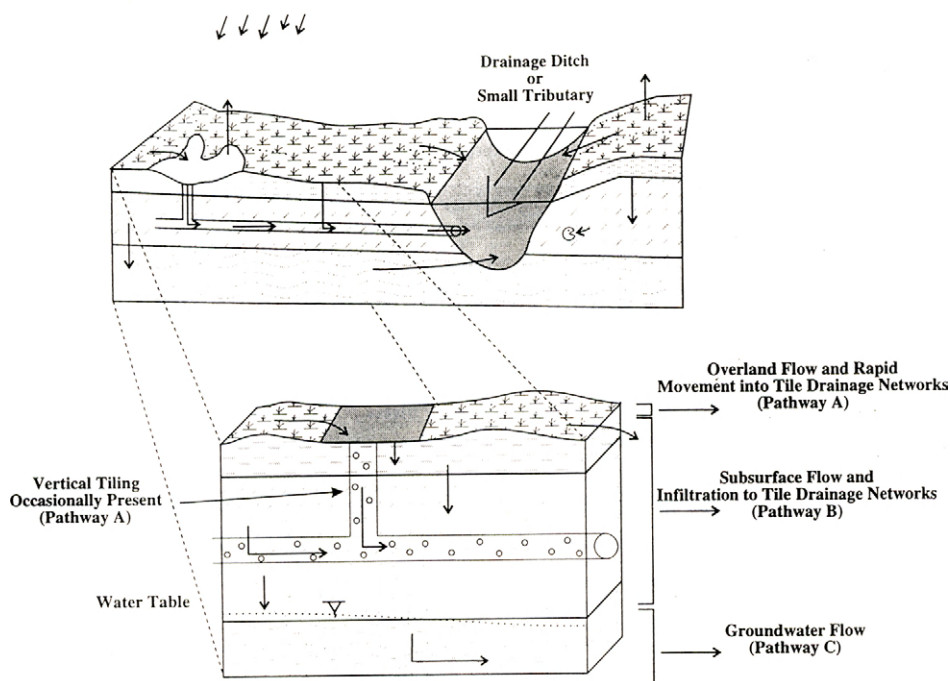


Fig. 1.5. Transport pathways of water and associated herbicides in agricultural fields. Three major mechanisms exist in the Minnesota river watershed: overland flow or runoff (pathway A), subsurface flow enhanced by networks of tile drains (pathway B), and leaching into the water table and transport with ground water flow (pathway C). Some of the networks have vertical risers to remove ponded water, flow through these rivers, or rapid infiltration to a horizontal tile line produces a discharge signal similar to overland flow [48].

system. Tile drainage networks have the potential of producing two different hydrological and chemical signals: a rapid response via routing through vertical tile risers and /or fast infiltration to horizontal tiles, or a delayed signal via slow percolation of water into buried tile networks.

In the above study, it was shown that the metabolite of atrazine, de-ethylatrazine, was present throughout the year. Half-lives of 21 and 58 days were calculated for 1990 and 1991, respectively. The longer conversion time in the 1991 soil was thought to result from rapid flushing from the soil and less exposure to soil microbes. In this respect, it should be noticed that the half-lives under real conditions can depend on the different mechanisms of transport of pesticides mentioned in Fig. 1.5 [48].

Another point to consider in the behaviour of pesticides is the so-called soil pore water. One of the more studied examples has been atrazine [49]. De-ethylatrazine and de-isopropylatrazine, major metabolites of atrazine, were found in the soil pore

TABLE 1.11

SOLUBILITY IN DISTILLED WATER AT 22°C, PARTITION COEFFICIENT (K_d) AND MELTING POINT FOR SEVERAL TRIAZINES

Compound	Solubility (mg/l)	Melting point (°C)	K_d [(ng/g)/(ng/ml)]
Atrazine	33	175	1.1
De-ethylatrazine	670	133	0.4
De-isopropylatrazine	3200	177	0.3

water. To assess their relative adsorption on soil, the partition coefficients of the three compounds have been determined, and are shown in Table 1.11. It can be seen that the order of retention in soils varies as a function of the alkyl chain-length, as de-isopropylatrazine < de-ethylatrazine < atrazine. Following these studies, it was found that the DAR (relation between concentrations of de-ethylatrazine to atrazine ratio) was higher in soil pore water than in soil cores sampled from the same depth. The greater mobility of dealkylated metabolites throughout the unsaturated zone, via soil pore water, may pose a potential threat to groundwater quality, especially in more porous soils.

Another topic of importance is the prediction of the transfer of pesticides into the aquatic environment. A list of physicochemical properties has been given in Table 1.10. A paper that has been much used for such studies is by Gustafson [50]. In this, a method has been proposed for 22 priority pesticides, which considers soil mobility and soil persistence to indicate which should leach and which should not. A particular combination of these two properties separates the leaching and non-leaching pesticides. The Gustafson expression, or GUS index, is given by

$$\text{GUS} = \log(\text{soil half-life})(4 - \log K_{oc})$$

When GUS is >2.8, the pesticide can be considered to be a leacher; for GUS higher than 1.8 and lower than 2.8, the pesticide can be considered a transition leacher, and for values lower than 1.8 the pesticide is considered to be a non-leacher to ground water. Typical values of the GUS index for common pesticides are indicated in Table 1.10.

Other papers have showed correlations between the soil sorption constants based on organic carbon, water solubility, octanol–water partition coefficient, retention-time in reversed-phase HPLC, and molecular weight [51]. In general, it has been shown that the chemicals with a low water solubility tend to adsorb to soil, those with a low vapour pressure tend to dissipate slowly from water, and those with relatively high octanol–water partition coefficients, or low water-solubility, have a high potential for bio-concentration [52].

The choice of different soil types with different characteristics such as pH, organic matter, particle-size distribution, and biomass, and which should be fully characterized, is needed. Studies of various soil types – and usually fresh soils, since storage or drying causes loss of part of their microbial degradation capacity – are needed. Similarly, studies are required on the choice of sediments, the different water types (i.e., surface, ground, river, estuarine and soil-pore water) and the pesticide formulations for use under different application conditions. These should provide a better understanding of the behaviour of pesticides in the aquatic environment.

1.3.1. Pesticides in soil

After application, a significant portion of the pesticides applied in agriculture is found to remain associated with the soil over long periods of time. It is important to ascertain the toxicity and stability of these chemicals in the soil environment. Soil itself plays a major role in determining the fate of chemical pollutants. In the soil, xenobiotics may be transformed by biotic or abiotic processes thus leading to pesticide transformation products.

The study of pesticide metabolism in soil, sediment and waste systems is of great importance from environmental, ecological and economic standpoints. Most of the studies during the last few years have focused on the kinetics of degradation of the parent molecules, some transformation products have also been characterized in many instances. The various factors related to the transformation of pesticides in soil have been reviewed [53].

The mobility of pesticides in soils is relevant to their leaching, volatilization and bio-availability to flora and soil. This mobility is largely dominated by sorption processes following a variety of mechanisms. These processes depend on the lipophilicity of the pesticide, the soil mineralogy and organic matter content, and especially the soil humidity. In general, it has been stated that a decreasing water content increases adsorption and reduces the mobility, increasing temperature reduces the adsorption and increase mobility, increasing clay-mineral content and organic-matter content increase adsorption and reduce the mobility, and that plant cover increases metabolism and reduces leaching. Some of these aspects are discussed in more detail below.

It is known that the *soil type* has a profound influence on the persistence of pesticides and their transformation products. The soil pH, for example, is an important parameter affecting the persistence of chemically unstable pesticides, especially those belonging to the organophosphorus and carbamate groups. The adsorption and mobility are interrelated, and the mobility depends essentially on the degree of interaction between the pesticide molecule and the soil components. The mobilities of acidic herbicides, organophosphorus pesticides, and atrazine are pH-related, with higher mobility in soils with higher pH [54].

In general, we can say that pesticides and their degradation products persist longer

in sandy soils than in organic-rich soils, and that the addition of rice straw accelerates the degradation of pesticides. Other aspects to consider, related to the soil type, are the differences in redox potential when soils are flooded, and the impact of flooding on pesticide-degrading microorganisms which might be responsible for such variations in the degradation pattern of pesticides in different soils. There is increasing evidence that chemically catalyzed interactions between the dominant redox system and pesticides or their degradation products may be common and widespread in anaerobic conditions such as flooded rice soils [55,56].

The *water regime* also affects the extent of degradation, the type of products formed, and the persistence of these product on soils. Usually, under flooded conditions, many organophosphorus (parathion, fenitrothion) and carbamate (carbaryl) pesticides are hydrolyzed. The degradation of the pesticides in soils is closely related to the redox potential following the flooding conditions. The redox status of most soils rich in active organic matter decreases within a few days after flooding. A recent study has been made on the pesticides trichlorpyr and benomyl, which are applied after the permanent flood on a rice field [57]. In general, their dissipation in the rice-paddy water was rapid, by hydrolysis. For carbofuran, one of the most important mechanisms was shown to be adsorption to the soil, and the data indicated that breakdown in soils is rapid at 2 and 20 cm depth, so ground water contamination for rice production is probably low. However, higher concentrations of carbofuran would most likely be found in the paddy water since its target pest residues are there, and it is applied in granular form. Thus its potential would be greater for surface water contamination than ground water contamination if the flood water from the rice field is not maintained within the levels of the field.

Organic matter and *temperature* are the other two parameters that essentially affect the formation of the transformation products of pesticides in soil samples. In general, by increasing the organic-matter content and the temperature, the degradation of pesticides in soils is enhanced. When the organic-matter content increases, the biomass of the active microbial population also increases and so does the degradation. The effect of temperature on degradation has been shown in different studies conducted in tropical ecosystems [58,59]. It is clear that the high temperatures encountered in the tropics will lead to faster degradation of pesticides, as has been shown for a few examples: fenitrothion, methyl-parathion, diazinon, carbaryl and carbofuran. For terbutylazine, it was also been shown that the half life varied from 38 days at 25°C to 14 days at 35°C, and also varied according to the soil type; it was always shorter in tropical regions [60].

The role of the organic matter in soils is very important. It has been shown that the most persistent complexes result from the direct covalent binding of pesticides to soil humic matter or clay. The pesticides most likely to bind covalently to the soil have chemical functionalities similar to the components of humus. The humic material is derived from the remains of decomposing plants, animals and microorganisms, and is composed primarily of humic and fulvic acids. These molecules are polymeric

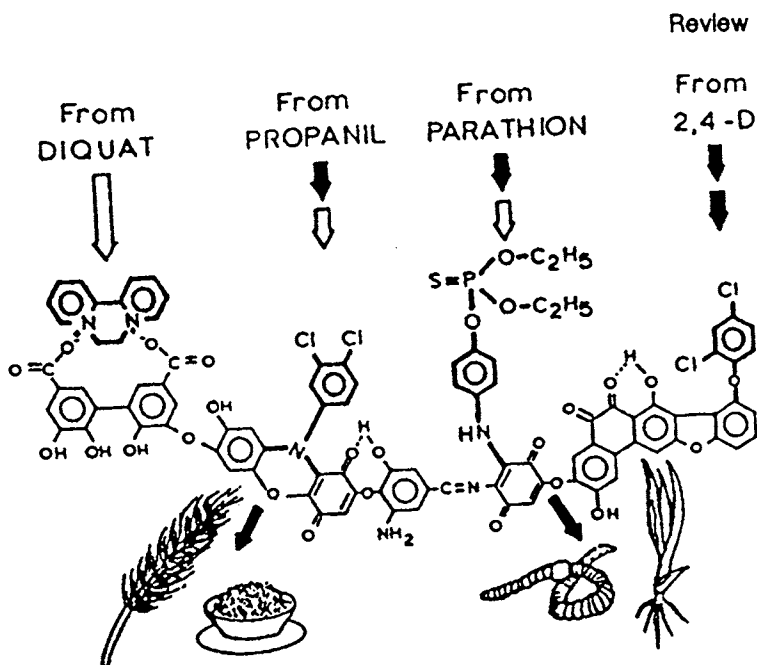


Fig. 1.6. Example of binding of different pesticides to humic substances in soil.

and consist of an aromatic moiety containing mono- di- and polyphenolic subunits. In fact, phenolic compounds account for up to 30% by weight of the humic polymer. It is important to note that humic substances occur not only in the soil fraction, but also in sewage effluents, peat, coal, and lignite, and also in river, marine, and lake sediments. Thus, pesticides that structurally resemble phenolic compounds can bind covalently to humus, and their complexes can be found in all of the above environments. Figure 1.6 gives an example of the binding of the various pesticide families to humic substances.

The *cation exchange capacity* is a further parameter to take into account, and was a significant predictor of the mobility of, e.g., diazinon and carbofuran. This capacity has been correlated negatively with the movement of pesticides and positively with adsorption [56].

The *clay content* should also be taken into consideration. Adsorption is often correlated with the clay content of soils and the mineral fractions of clay play an important role in adsorption. Organic matter has a stronger effect, but the adsorption of pesticides on to clay is important because must soils contain much more clay than organic matter, and the clay contributes significantly to the cation-exchange capacity. The influence of clay on pesticide adsorption and mobility is more evident in subsoils, which are rich in clay and poor in inorganic matter [54].

In order to investigate the soil degradation of pesticides, laboratory incubation

studies with ^{14}C -labelled pesticides are required. These allow one to assess the likely rate of degradation of parent pesticides in soil, and provide information on the structure and likely degradability of metabolites. The studies should also assess the rate of mineralization of the pesticides by monitoring the rate of formation of $^{14}\text{CO}_2$. Traps should also be included in the apparatus to retain any other volatile compounds released from the soil. The ^{14}C mass balance resulting from these studies is an important element of the hazard-assessment process. Degradation under aerobic conditions is of primary interest. Testing under anaerobic conditions may also be important in certain cases, to observe reductive reactions, e.g., with pesticides which penetrate deeply into the soil profile or which are used in paddy rice. Incubation with sterilized soil is sometimes carried out to determine the extent to which chemical mechanisms participate in the degradation of pesticides, and is also of interest.

1.3.1.1. Atrazine degradation in soil

One of the pesticides whose degradation in soil samples has been much studied is atrazine. An interesting paper has been published on the persistence and degradation of ^{14}C atrazine in soil [61]. The degradation pathway of atrazine in soil is shown in Fig. 1.7. This was investigated under saturated and unsaturated conditions with samples of soil taken from four depths down to 120 cm. It was shown that under saturated conditions under 90–120 cm depth, the half-life of atrazine was 87 days. De-isopropylatrazine was less persistent in saturated soils with a half life of 58 days. The increased degradability of de-isopropylatrazine as compared to atrazine, and the reduced persistence of de-isopropylatrazine under saturated soil conditions, provide a logical explanation for its relatively low concentration in ground water, compared to atrazine. In this context, it was shown that de-isopropylatrazine was more susceptible to degradation than atrazine, in the top soil; the persistence of both compounds increased at greater depths. The microbial degradation of atrazine to de-isopropylatrazine is less favoured than that to de-ethylatrazine. The lower degradability of de-isopropylatrazine than of atrazine, and the reduced persistence of de-isopropylatrazine under saturated soil conditions might also explain the very low concentrations of de-isopropylatrazine detected in ground water, as compared to atrazine and de-ethylatrazine. In this context, it has been shown [62] that the atrazine ring and its isopropyl side chain were mineralized more slowly under denitrifying conditions than under aerobic conditions in soil systems. Oxygen limitation at greater soil depths will retard the atrazine transformation and mineralization as the soil environment becomes more anoxic. The limitation is that if atrazine or its metabolites are transported into the deeper soil and ground water where denitrifying conditions may prevail, mineralization and the transformation of atrazine and its metabolites will be greatly reduced. The slow biotransformation pathway for atrazine and de-ethylatrazine is dealkylation by loss of the isopropyl side chain and this, in combination with denitrifying conditions, may account for the frequent detection of atrazine and some of its metabolites, especially de-ethylatrazine. Also, at greater soil depths the

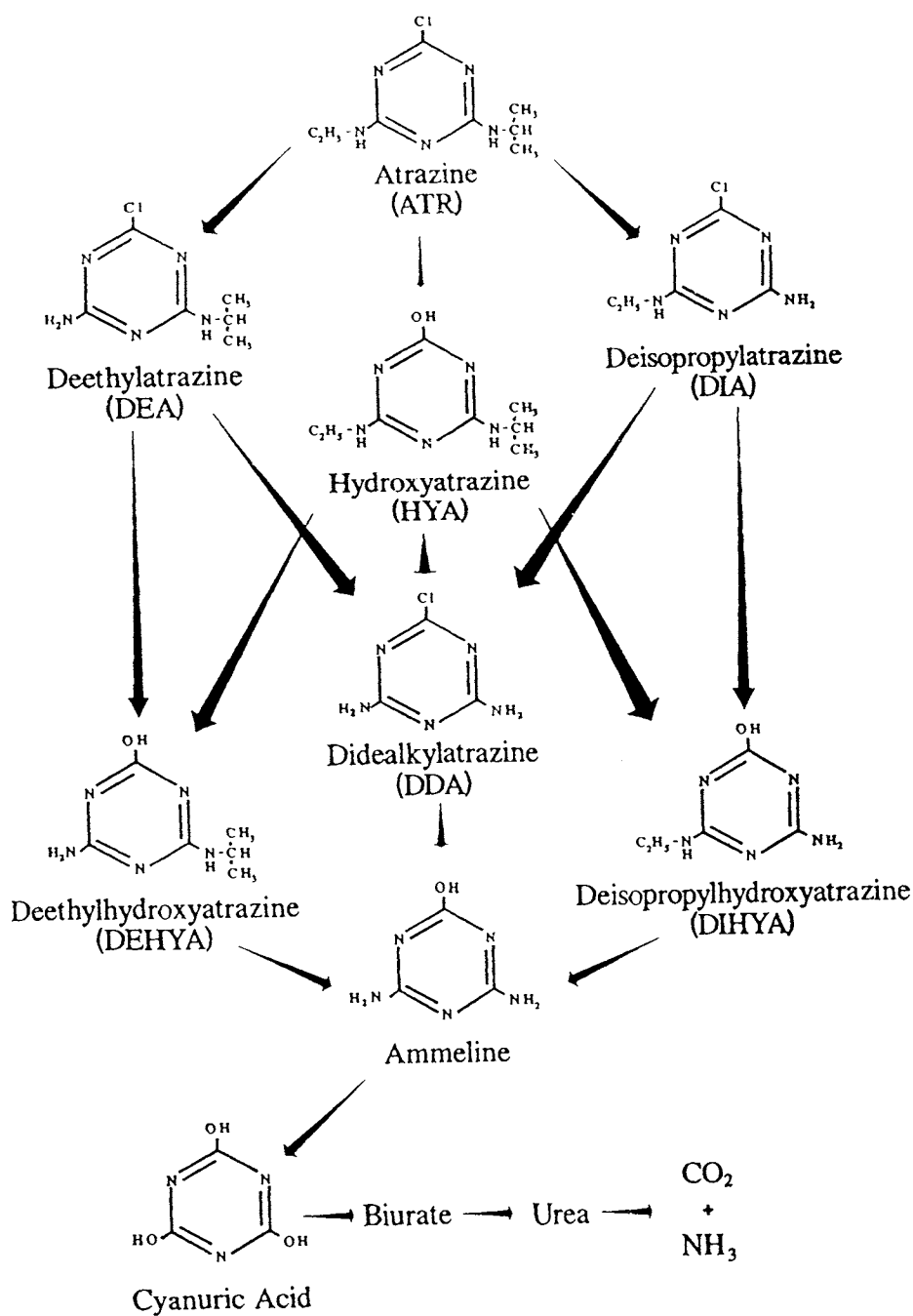


Fig. 1.7. Degradation pathway of atrazine in soil.

microbial activity will be much lower with lower organic carbon, thus retarding the biotransformation processes even more. The slow dealkylation by loss of the isopropyl side chain of atrazine, and its even slower mineralization under anoxic conditions, will have implications for bio-remediation of sites contaminated with atrazine. Similarly it was shown [63] that, under field conditions, atrazine, simazine and deisopropylatrazine undergo preferential removal of an ethyl rather than an isopropyl side chain in the unsaturated zone. Furthermore, the dealkylation rates for atrazine and simazine are comparable and approximately 2–3 times more rapid than those of de-isopropylation of atrazine and propazine. This indicates that the removal of an ethyl side chain is preferred over that of an isopropyl side chain, regardless of the nature of the parent triazines. Further dealkylation of mono-dealkylated metabolites at depth in the unsaturated zone shows preferential removal of ethyl rather than isopropyl side chains.

The degradation of atrazine in a deltaic rice soil is shown in Fig. 1.8 [64]. From this figure it can be seen that: the half life is ca. 1 month: and de-ethylatrazine is always detected to some extent with a ratio of de-ethylatrazine/atrazine of 0.1–0.3. Also, there is permanent soil contamination of the Ebro delta area by atrazine, which corresponds to a value of 20–25 ng/g, approximately 8% of the level detected when atrazine has been freshly applied (310 ng/g).

The degradation of the herbicide atrazine in soils has led to numerous investigations. Three different factors affect the process: biological degradation, chemical degradation and volatilization. The conversion of atrazine to its main metabolite, de-ethylatrazine, results primarily from the metabolic activity of soil bacteria and fungi [65]. The formation of de-ethylatrazine as the main metabolite from atrazine has

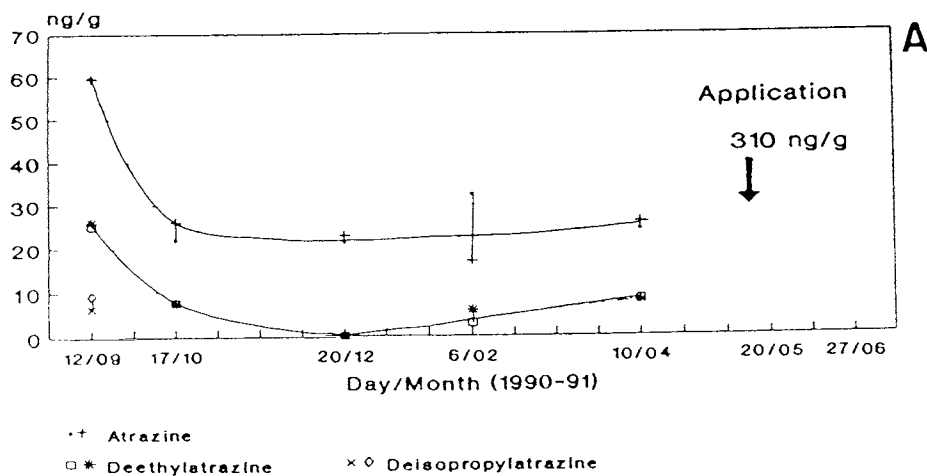


Fig. 1.8. Degradation of atrazine in a deltaic rice soil.

been reported in a number of papers dealing with its behaviour in soil samples [66,67].

Chemical degradation by processes such as hydrolysis appear to be more important than biological degradation in sandy soils with organic carbon content up to 4.3% [68]. It has been reported that the concentration of atrazine in the same type of soils is consistent with the soil's organic matter and clay content, with the organic matter trying to immobilize atrazine [69]. Volatilization of atrazine seems to be of minor importance compared to the other degradation pathways. A report showed volatilization of atrazine in a soil from Maryland to be negligible, with only 2.4% loss after a 21-day period, indicating that such losses were much smaller than disappearance by chemical degradation [70]. This can be explained by the vapour pressure value of atrazine which is 0.00004 Pa, much lower than that of fenitrothion (0.0072 Pa) [41]. Consequently, fenitrothion losses in Ebro delta soils, where it is currently applied, are reported to be caused mainly by volatilization [71].

Under the real environmental conditions of the Ebro delta area, gave a half life for atrazine in the range reported in the literature. Values of 50 [68], 21.5 [72,73] and 28 days [74] have been reported in soils with similar characteristics to that soil studied in our work. Although volatilization does not play an important role in the degradation of atrazine from soil samples, it does affect the half lives when the temperatures vary between winter and summer periods. Thus the half-life varied between 19 and 60 days, depending on the year and season of applications in Maryland [75], and from 37 to 125 days when the dissipation period covered either the summer (up to 20°C) or the winter periods (down to -16°C) [66].

For the above reasons, it can be considered that the degradation of atrazine in the Ebro delta soil is caused mainly by chemical degradation since the soil contains a low organic-matter content, below 1% [68,69]. Since most of the sampling has been carried out during the winter season losses by volatilization are negligible.

Another aspect to be considered is the so-called permanent contamination by atrazine in the soil. It has been reported that levels of atrazine remaining 238 days after application were 10 ng/g (i.e., 1.5% of the level applied) pesticide remaining after 238 days of application [72]. It has also been reported that on a soil containing 25 ppm of atrazine on the day of application, 30% remained 1 year after incubation. This amount corresponded to atrazine, de-isopropylatrazine, de-ethylatrazine and hydroxyatrazine, and was attributed to bound residues. The formation of such bound residues may be linked with the chemically stabilizing reactions between phenolic-OH and -COOH groups of soil organic matter and the pesticide and/or metabolites. It has been suggested that humic materials consist of phenolic and benzene-carboxylic acids joined by hydrogen bonds to form a molecular-sieve type of polymeric structure of considerable stability. One of the characteristics of this proposed structure is that it would contain voids or holes of different molecular dimensions which could trap atrazine and /or its degradation products. It should be added that the application of this structural concept to the bound residues of pesticides and their metabolites in

organic matter or humic material is still a matter of conjecture [67]. Other authors have suggested a slightly different theory for such a permanent contamination, which is related to the mode of adsorption of atrazine turning slowly from physical to chemical adsorption. It may be that the basic compounds adsorbed onto the silicate surface migrate slowly from certain adsorption sites where they are chemisorbed via salt formation [75].

In the data on atrazine degradation shown in Fig. 1.8, it can be seen that the permanent contamination of the Ebro delta soil by atrazine corresponds to levels of 20–25 ng/g, which is ca. 8% of the herbicide applied and is similar to values found by other authors [76]. The level of de-ethylatrazine is usually below 10 ng/g. When considering these levels, we should mention that there is a continuous treatment of soil with atrazine, which has resulted in permanent contamination of the soil surface. According to model programs, atrazine percolation in a soil takes approximately 2 years [77] and so the same period should be required for complete decontamination of the Ebro delta soil.

It has also been shown [64] that although atrazine exhibits a half life of ca. 30 days under the Ebro delta conditions, a permanent level of 20–25 ng/g of atrazine has been obtained. This permanent level of contamination has been attributed to the organic fraction of the soil which appears to have a potential for forming bound residues. In this sense it is suggested that atrazine and de-ethylatrazine are retained by humic materials by a process involving adsorption on external surfaces and entrapment in the internal voids of a molecular-sieve-type of structural arrangement. In the future, it will be important to obtain more information about the mechanisms of binding of pesticides and their degradation products in the soil.

In conclusion, we should point out that the study of the soil is not the main topic of the present book. The case study of atrazine has been given as an example, but there are many cases in the literature of soil dissipation studies and of the behaviour of pesticides in soil. We are more interested in the fact that after the herbicides are applied to the soil surface, they can leach through the ground and also to the surface waters by a variety of processes. These aspects are now discussed in more detail.

1.3.2. Pesticides in ground waters

A general scheme of the zones of subsurface water is shown in Fig. 1.9.

The data required to determine the environmental fate and potential of a pesticide to reach ground waters include information on its hydrolysis, aquatic metabolism, leaching, and field dissipation. According to Ref. [78], a pesticide can reach ground water if its water solubility is greater than ca. 30 mg/l, its adsorptivity (K_{oc}) is below 300–500, its soil half-life is longer than ca. 2–3 weeks, its hydrolysis half-life is longer than ca. 6 months, and its photolysis half-life is longer than 3 days. The Office of Pesticide Programs (OPP) of the US EPA have evaluated approximately 800 active ingredients of pesticides in an effort to screen out those with the potential to

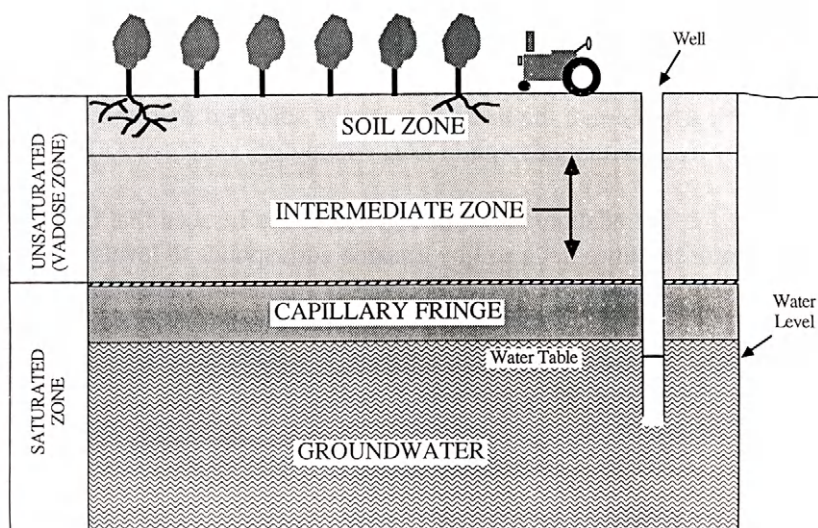


Fig. 1.9. Zones of subsurface water.

leach in ground water. The pesticides were screened on the basis of broad criteria related to the mobilities and persistence of the compounds listed in Table 1.12 [79]. If a pesticide was believed to be persistent and mobile, relative to these criteria, it was selected for further evaluation.

The potential of a pesticide to contaminate ground waters can also be assessed from the field conditions denoted by the acronym DRASTIC. This is an environmental index which includes seven key hydrogeological factors, namely, the depth to the water table, the recharging of ground water by precipitation and irrigation, the aquifer medium, soil medium, topography (slope), impact of the vadose zone, and

TABLE 1.12
PHYSICAL AND CHEMICAL CHARACTERISTICS OF PESTICIDES FOUND IN GROUND WATER

Characteristic	Leaching criteria
Water solubility	>30 ppm
Henry's Law constant	$<10^3 \text{ Pa (m}^{-3} \text{) (mol}^{-1} \text{)}$
Hydrolysis half-life	>25 weeks
Photolysis half-life	>1 week
Soil adsorption: K_d	$<1-5$ (usually $<1-2$)
Soil adsorption: K_{oc}	$<300-500$
Aerobic soil metabolism half life	>2-3 weeks
Field dissipation half life	>2-3 weeks
Depth of leaching in field dissipation	>75-90 cm

hydraulic conductivity of the aquifer. A DRASTIC score greater than 150, recharge values larger than 25 cm/year, and thin soils with high nitrate levels (a few ppm of natural background), are indicative that pollution of the ground water by pesticides is likely [78]. Koterba et al. [80] have reported the application of DRASTIC to pesticides in shallow ground water in the Delmarva Peninsula.

They concluded that the DRASTIC indices indicate that the shallow ground water in a region is generally vulnerable to contamination. The area- and depth-distribution of pesticide residues in ground water could depend chiefly on the patterns of land use, the associated pesticide use, the soils, and the shallow ground water flow. However, the DRASTIC method appears to be limited regarding pesticide movement. Consequently, instead of using DRASTIC, most potentially contaminated areas in the Delmarva Peninsula can be broadly targeted by first identifying leachable pesticides that are used at moderate to high rates and have a wide geographic use in the studied area. Then mapping the use of pesticides in relation to fine-scale data on associated crop types, and on naturally or artificially well-drained areas, provides a combination of features which were shown to relate to areas of shallow ground water contaminated by these pesticides under normal use.

One of the key parameters relating to ground water pollution via soils is the mobility (leachability) of the pesticide, which in turn is determined by such factors as K_{oc} , and the soil half-life and hydrolysis half-life. According to Ref. [50], atrazine, aldicarb, picloram and diuron are most liable to leaching. The types of prevailing soil and water affect pesticide degradation, particularly by abiotic degradation processes (e.g., hydrolysis and photolysis).

The European Union has recently proposed a priority list (Table 1.13) of pesticides that can leach or not, based on a consideration of their physicochemical properties (as shown in Table 1.12) and the amount used. These pesticides can be found in ground waters of the EU. Following the three general parameters (toxicity, persistence and input) used for selecting the priority list of pollutants in the UK, a red-list of substances was established that includes several pesticides, most of them common to the EU list. In order to prevent the contamination of ground and drinking water by pesticides in Europe, the priority list considers pesticides of which over 50 tons per annum are used (those over 500 tons are underlined) and their capacity for probable or transient leaching [1]. Many of the pesticides indicated in Table 1.13 are also the more widely used (see also Table 1.4). There are a few other pesticides, such as demeton-*S*-methyl, fentin acetate, mancozeb, propineb, thiobencarb and zineb, which although they are used in amounts over 50 tons per annum, have insufficient data available to allow evaluation of the probability of leaching. Consequently these are not included in the table. Glyphosate and thiram were also not added because large differences in the ground water ubiquity score (GUS) index were found.

It is estimated that ground water is the source of drinking water for 90% of rural households and three-quarters of US cities. More than one-half of US citizens rely on ground water for their everyday needs. Owing to the amount of information indicat-

TABLE 1.13

PESTICIDES USED IN EUROPE IN AMOUNTS OVER 50–500 TONS PER ANNUM WHICH WERE CLASSIFIED AS PROBABLE OR TRANSIENT LEACHERS

<u>Alachlor</u>	Dinoseb	<u>Methabenzthiazuron</u>
Aldicarb	<u>Diuron</u>	Methiocarb
Amitrole	DNOC	Oxydemeton methyl
<u>Atrazine</u>	EPTC	Phenmedipham
<u>Benazolin</u>	Ethofumesate	<u>Prochloraz</u>
<u>Bentazone</u>	Ethoprophos	Propham
Bromofenoxim	Fenamiphos	Prometryn
<u>Carbaryl</u>	Fluoroxypyr	<u>Propiconazole</u>
Carbendazim	Iprodione	<u>Pyrethrin</u>
<u>Carbetamide</u>	<u>Isoproturon</u>	Simazine
<u>Chloridazon</u>	Linuron	Terbutylazine
Chlorpyrifos	<u>Maneb</u>	Terbutryn
<u>Chlortoluron</u>	<u>MCPA</u>	Triademinol
Cyanazine	<u>MCPP</u>	Trichlorfon
2,4-D	Metamitron	<u>Trichloroacetic acid</u>
Dalapon	<u>Metazachlor</u>	Vinclozolin
Diazinon	<u>Metham-sodium</u>	Ziram
Dichlobenil	<u>Metolachlor</u>	
<u>Dimethoate</u>		

ing the presence of pesticides in ground water in the US states [81], a joint research project between the Environmental Protection Agency (EPA)'s Office of Drinking Water (ODW) and the Office of Pesticide Programs (OPP) conducted a statistically based survey of pesticide contamination of drinking water wells. During the National Pesticide Survey (NPS) (see Table 1.8) 1349 drinking water wells were sampled and analyzed for 101 pesticides, 25 pesticide transformation products (TPs) and nitrate, with a total of 127 analytes. The results of the NPS were released in November 1990 (Phase I) and January 1992 (Phase II). The selection of the analytes was based on a use of at least 1 000 000 lb in 1992, water solubility greater than 30 mg/l, and a hydrolysis half-life longer than 25 weeks. Pesticides and pesticide degradation products previously detected in ground water, and the pesticides regulated under the Safe Drinking Water Act, were automatically included in this priority list. There have been many studies on the levels of pesticides in ground waters, in the US mainly [82,83], many of them related to triazines, and to insecticides such as carbofuran, carbaryl, chlorpyrifos, dimethoate and deltamethrin. From 105 collected ground water samples, 47% contained residues of pesticides. Surprisingly, carbofuran was one of the pesticides found at the higher concentrations, at 1 µg/l. However, few studies have been performed on the behaviour of carbamates in ground waters. As a case example, we discuss one area, with the monitoring of various wells during a 1-year period.

1.3.2.1. Carbamate pesticides

There is considerable information on the non-point source pollution of surface- and ground waters by herbicides such as triazines, amides and phenylureas [84–86]. However, hardly any data can be found on the ability of other pesticide families, such as the carbamate insecticides, to contaminate ground waters, or about their fate in aqueous environments. Carbamates are of environmental concern because of their high acute toxicity (e.g., aldicarb and carbofuran exhibit an LD_{50} in rat of 1 and 8 mg kg^{-1} , respectively). Some are suspected carcinogens and mutagens [87]. Generally, compounds which have a relatively high mobility and exhibit rather long soil half-lives can be transported into the ground water, by runoff (irrigation and/or precipitation). According to the GUS scale index, carbamate insecticides are classified as potential leachers because of their high water solubility. Despite their widespread use (e.g., for carbaryl, over 500 tons per annum in the European Union) it is quite surprising that ground water monitoring surveys have so far only given a few carbamate positives. In monitoring within the US, no aldicarb residues were detected in 100 wells sampled [88]. Only 4 wells out of 58 were shown to be carbofuran-positive in a monitoring undertaken in Central Maine (US), while carbaryl residues ranged from undetectable to 38 ng/l in rivers of Northern Greece [89,90]. The TPs of carbamate pesticides are generally more toxic than the parent compounds; e.g., 1-naphthol has been found to be more toxic to aquatic organisms than is carbaryl, its parent compound [91].

Therefore, there is a need to include in monitoring programmes the main polar carbamate TPs, as recommended by the National Pesticide Survey and as can be seen in Table 1.8 [92]. This will provide the information about their threat to ground waters.

A case study has been recently reported for Almeria, which is a Spanish province located in the Southwest of the Mediterranean coast [93,94]. With 36 460 hectares of cultivated fields, mainly greenhouses, this region has become one of the major suppliers of green vegetables to most of the EU countries. The major crops grown include peppers, tomatoes, melons, and cucumbers; total production reached 1 384 240 tonnes during the 1992–1993 season. With a hot and dry climate (average maximum temperature 24.2°C, and an average annual precipitation of 300 mm) large amounts of insecticides and, to a lesser extent, fungicides are sprayed to improve crop yields. Herbicides are used in very low amounts for weed control, as a consequence of the regular sprinkler or furrow irrigation. Figure 1.10 shows the area of Almeria (South East Spain) where the well water monitoring took place.

The soil is predominantly silt; loamy with value near 60%. The clay content at the surface is 20% but it tends to increase with the depth. The organic-matter content averages 2% in the 1–50 cm surface layer, but falls rapidly with depth to a very low level. The Almeria soil has the ability to adsorb pesticides and has a rather high hydraulic conductivity; consequently pesticide movements can be expected. The soil pH is very similar between samples ranging from 7.5 to 8. One of the major factors

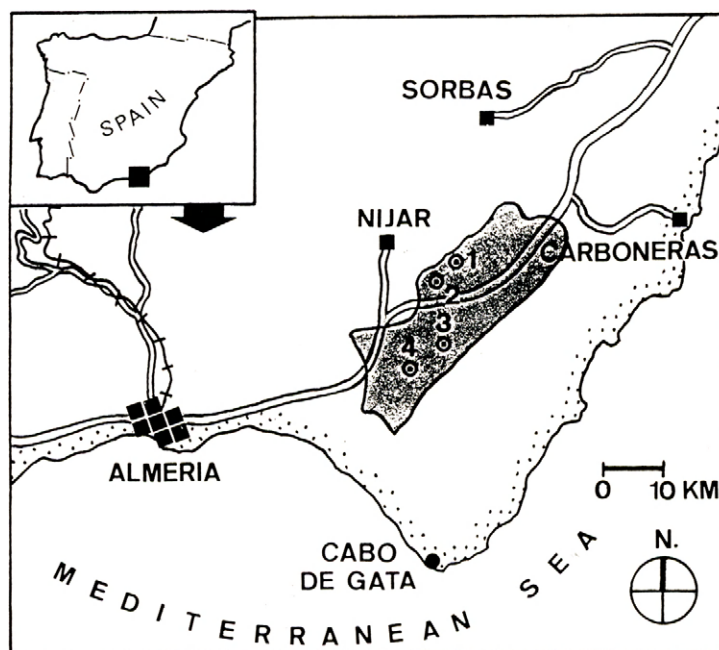


Fig. 1.10. Almería area with the monitoring of the different wells.

affecting leaching is the water recharge rate (rainfall plus irrigation minus evapotranspiration). The extensive use of irrigation has been one of the reasons for the movement of agrochemicals in the soil. The amount of deep percolating water produced by irrigation can be related quantitatively to the leaching capacity of pesticides [95], and irrigation schedules can dramatically affect the downward movement of water soluble pesticides. In addition, the extensive use of greenhouses in this area would promote more rapid degradation of residues resulting from increased soil temperatures. In this respect, the agricultural practices have affected the Almería ground water quality. The total pesticide pollution exceeded the maximum contaminant level for drinking water ($0.5 \mu\text{g/l}$), allowed by the EU Drinking Water Directive twice a year, in the summer (June, July) and the late winter (February, March), as shown in Fig. 1.11.

The overall pollution behaviour is similar in each of the four wells despite a great variation in the hydrological conditions through the aquifer. The lack of spatial variation within the aquifer can be explained by the low flow within it, which results from its over-exploitation for irrigation purposes. The carbamate insecticides methiocarb, methomyl, and carbofuran and the triazine herbicides, simazine and atrazine were the main pollutants. Seasonal variations showed different environmental behaviours for the two pesticide families. The triazines are chemically stable and contamination levels of $0.04\text{--}0.2 \mu\text{g/l}$ were detected throughout the year. These

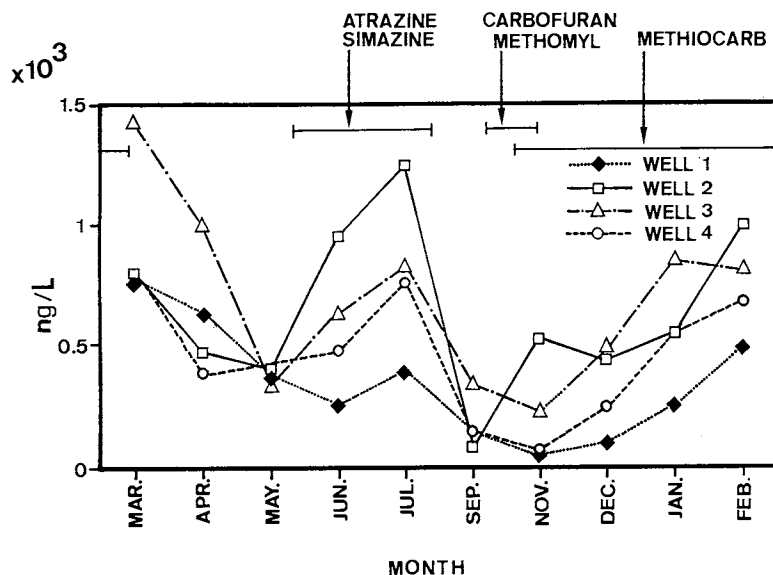


Fig. 1.11. Seasonal pollution in well waters from the Almería.

compounds exhibited a conservative behaviour in ground water. In the anaerobic conditions of an aquifer, the microbial activity is very low and atrazine degrades very slowly [65]. De-ethylatrazine, the major TP of atrazine, was only detected in the last 2 months of the monitoring in Wells 2 and 3 (results not shown) at a concentration level of $0.1 \mu\text{g/l}$. In general, the conversion of atrazine into de-ethylatrazine is a preferential degradation process in the unsaturated zone. A higher mobility for de-ethylatrazine than atrazine in the soil-pore water, is expected, mainly because of its higher water solubility (670 versus 33 mg/l , respectively) (see Table 1.11). A reasonable explanation for these observations is that the levels of de-ethylatrazine found correspond to the atrazine applied several weeks or months before, whereas the levels of atrazine are caused by point-source pollution. The pollution by triazines was not homogeneous throughout the aquifer and the lack of atrazine in Well 4 was taken as emphasizing the point-source-contamination origin hypothesis. Although the amounts of triazines used could not be estimated in this particular area, they are surely very low with respect to the insecticide and fungicide consumption as a consequence of cultivation practices.

The carbamate insecticides account for a higher contribution to the pollution with, for example, a concentration level of $0.8 \mu\text{g/l}$ of methomyl in March 1993 in Well 3 but they are not stable in the hydrological environment. Methomyl is usually sprayed in September–October and appeared in the ground water in February, with a maximum contamination peak in March. Carbofuran and methiocarb are less water-soluble than methomyl and leached to ground water by the sixth month after appli-

cation, with a maximum contamination peak in June–July. The persistence of pollution by carbamate insecticides has been estimated to be in the range of 3–4 months. Their fate in ground water itself is linked to a hydrolysis pathway, and laboratory stability studies using slightly basic distilled waters indicated that methomyl, carbofuran and methiocarb remained stable for 60, 45 and 30 days, respectively. Pesticide adsorption on particulate matter was regarded as being a minor transformation pathway because of the compound's high water solubility. The striking feature was that carbamate leaching occurred very quickly in response to winter and spring furrow irrigation (3 months for methomyl, and 6 months for carbofuran and methiocarb). A rapid transport of carbamate insecticides through the soil may be explained by a macropore flow through the deeper soil core connected directly to the upper layer of soil (20–30 cm). This vertical channel route acts to bypass the soil matrix and permits a rapid leaching to ground water to occur preferentially in non-tillage soils such as those of the Almeria area [96]. Carbaryl and butocarboxim were found occasionally and seemed not to be a real problem for ground water quality. The former compound is used together with organophosphorus pesticides whereas the latter is sprayed to improve citrus crop yields.

One of the most important aspect of the study performed in the Almeria area concerned the carbamate transformation products. Methiocarb sulfone and 3-hydroxycarbofuran, the main degradation products of methiocarb and carbofuran, respectively, were detected in real ground waters for the first time and their concentration fluctuations followed very closely those of the parent insecticides (see Fig. 1.12). The loadings of carbofuran and methiocarb in the aquifer during the year following their application looked suspicious. The most likely hypothesis is that both insecticides were adsorbed onto the Almeria soil, metabolized in the unsaturated soil layer, and slowly transported from the overlying unsaturated zone into the aquifer in response to seasonal irrigations. Once they have reached the deeper layers of soil, lower degradation rates can be expected because the carbon content is becoming

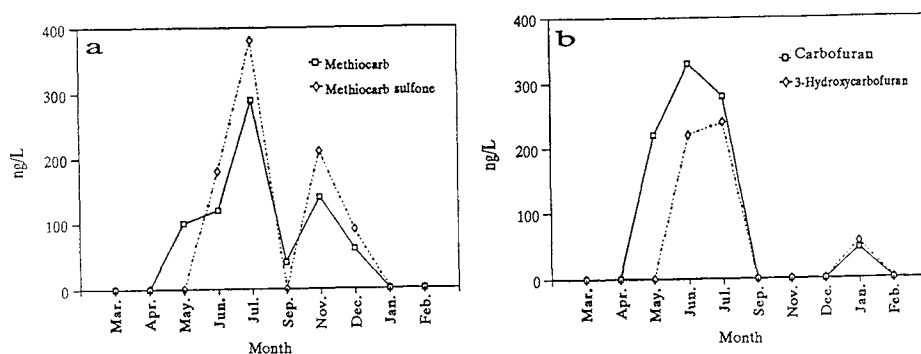


Fig. 1.12. Pesticide transformation products in Almeria ground waters.

smaller, as was observed for aldicarb's degradation in soil [97]. Carbofuran was used as a soil treatment, with 3-hydroxycarbofuran resulting from metabolic degradation. In contrast, methiocarb was foliage-sprayed and the question was whether the methiocarb sulfone appeared as a result of metabolic activity of soil bacteria and fungi, or as a sunlight-induced photo-oxidation process before reaching the upper soil layer. Photo-degradation processes only lead to the formation of methiocarb sulfoxide as an intermediate compound. Further irradiation brings about loss of the sulfur moiety. Additionally, foliage-residue studies indicated that only methiocarb sulfoxide is formed as a main metabolic breakdown product. Methiocarb sulfone can be regarded as a result of microbial oxidation in the unsaturated soil layer, and not a result of an abiotic process such as photolysis.

The fact that important levels of methiocarb sulfone and 3-hydroxycarbofuran are found in an aqueous environment indicates that both metabolites are more water-soluble than their parent compounds. Also, a quicker leaching in greater amounts can be expected in the underlying aquifer, in the same way that the concentration of de-ethylatrazine exceeded that of atrazine in the aquifer 15 weeks after application. The lower stability of the degradation products relative to the parent compounds can explain the fact that the metabolite concentrations have never exceeded those of the parent compounds in the monitoring programme. Although carbamate insecticides are well known for their easy degradation in aqueous environments, a few, methomyl, methiocarb, and carbofuran, were found incidentally in high concentrations (above $0.5 \mu\text{g/l}$) in a representative Almeria aquifer. Their discovery has been a source of concern because of their high acute toxicity but, fortunately, they are not stable in slightly basic aqueous environments and their persistence has never exceeded 3 months.

The identification of new metabolites is gaining importance, as is shown by the identification of new metabolites of alachlor [9,98]. Ground water samples collected in Massachusetts corn fields showed that more than 20 compounds could be identified as derivatives from alachlor, presumably via environmental degradation. Most of these were diethylaniline derivatives and their concentrations ranged from 4 to 570 ng/l [98]. Whilst analyzing 303 wells across the US Midwest, it was shown that alachlor ethanesulfonic acid metabolite was detected as frequently and very often at much higher concentrations than its parent compound, alachlor. Other metabolites detected were cyanazine amide – almost twice as frequently as the parent cyanazine. The results show that it is necessary to have information on pesticide metabolites in order to understand their environmental fate. As a consequence, the authors pointed out [9] that if pesticide metabolites are not quantified, the effects on ground water quality of chemical use will be substantially underestimated. It was concluded in that study that continuous research is needed to identify major degradation pathways for all pesticides, and to develop analytical methods to determine their concentrations in water and other matrices. This area will continue to expand since efforts for pesticide detection in the environment will be directed towards the development of methods

for very polar pesticide analytes, such as sulfonic metabolites of alachlor, or hydroxylated or carboxylic metabolites. The use of liquid chromatography-mass spectrometry with high-flow pneumatically assisted electrospray has permitted the identification of hydroxylated metabolites from bentazone in environmental waters [99]. The use of this technique opens up new possibilities for the trace determination of polar pesticide metabolites in water matrices and will probably facilitate a better understanding of fate of pesticides in the aquatic environment.

1.3.3. Pesticides in river and estuarine waters

The current generation of pesticides is not as fat soluble as the organochlorinated pesticides, such as DDT. They are transported in aquatic systems primarily in the dissolved phase, generally exhibit much shorter half-lives than the organochlorinated pesticides, and have only a minor bioaccumulation potential. This is true for the agricultural use of herbicides, which are being used in increasingly larger amounts than insecticides. In the last few years, the monitoring programmes have started to consider the new generation of pesticides and their transport through rivers and estuarine areas.

Agrochemicals are applied annually to agricultural soils throughout the world. Many of these organic chemicals are transported to surface waters by a variety of mechanisms such as non-point source pollution, ground water discharge, or atmospheric deposition. Millions of pounds weight of relatively water-soluble pesticides, such as atrazine, simazine, alachlor and metolachlor, are applied each year in the USA and in Europe as pre- and post-emergence weed control agents on crops such as corn and soybean [2,100]. It is reasonable to assume that substantial amounts of these compounds may be present in the surface waters which drain agricultural areas of each country. Also, highways and railroads close to rivers may receive the impact of non-agricultural applications of pesticides, such as triazines, chlorinated acids, and phenylureas.

Following considerations based on usage information, physico-chemical properties, and persistency, a priority list of herbicides was established for the Mediterranean countries France, Italy, Greece and Spain. The list, shown in Table 1.14, considers selected herbicides and fungicides that can cause contamination of estuarine and coastal environments. The selection of pollutants has been based on the availability of usage data and the consideration of half-lives, so pesticides that do not exceed a total of 10 tonnes after 90 days of application have been excluded. Fungicides such as carbendazim, ethirimol, metalaxyl, captan, folpet, captafol, vinclozolin and chlorothalonil, which are each used in amounts varying from 20 to 300 tonnes per year in the cited Mediterranean countries are also included. Other fungicides such as mancozeb, maneb, ziram and thiram, which are also used in the same countries but in larger amounts, between 300 and 700 tonnes per year, were not included in a pilot monitoring study because of the need to develop specific methods for analysis at the low $\mu\text{g/l}$ level in water samples [101].

TABLE 1.14

HERBICIDES AND FUNGICIDES OF POTENTIAL CONCERN IN THE MEDITERRANEAN REGION

<i>Herbicides</i>		
Alachlor	EPTC	Molinate
Amitrole	Ethalfuralin	Napropamide
Atrazine	Ethofumesate	Neburon
Bentazone	Flamprop-M-isopropyl	Paraquat
Bromoxynil	Glyphosate	Pendimethalin
Butylate	Isoproturon	Phenmediphan
Carbetamide	Linuron	Prometryn
Chlortoluron	MCPA	Simazine
2,4-D	Mecoprop	Trichloroacetic acid
Di-allate	Metamitron	Terbumeton
Dichlobenil	Metazachlor	Terbuthylazine
Dichlofop-methyl	Metabenzthiazuron	Terbutryn
Dinoterb	Metobromuron	Tri-allate
Diquat	Metolachlor	Trifluralin
Diuron	Metoxuron	
DNOC	Metribuzin	
<i>Fungicides (IAEA)</i>		
Captafol	Mancozeb	
Captan	Maneb	
Carbendazim	Metalaxyl	
Ethirimol	Vinclozolin and chlorothalonil	
Folpet	Ziram and thiram	

1.3.3.1. Organonitrogen pesticides in river waters

Some of the most relevant information on the environmental behaviour of pesticides in aquatic systems is the extent to which, after application, they can reach surface and river waters and, at a later stage, estuarine waters, with the potential of contaminating coastal sea waters.

The fate of chlorotriazine herbicides in aquatic systems has occasioned several investigations in recent years. The modelling approach, under laboratory conditions, has established that around 92% of the herbicide will be in the dissolved phase whereas about 1% is in the particulate matter [68,100].

Field studies have agreed with the modelling approach, giving values of 99.5% and 0.5% of atrazine in the dissolved and particulate matter, respectively. Atrazine has been transported in a number of river basins with an estimated loss of the applied herbicide varying between 0.4 and 1.8% [48,100,102]. These values have been reported from monitoring in the Camargue region, on the Rhone estuary [103] where the concentration values from the different stations are the same order and agree with the concentration range expected from other rivers such as the Mississippi in Minne-

sota and others. The concentrations in the dissolved phase were ca. 95–97% whereas in the particulate matter they vary between 3 and 5%. This behaviour can be explained by the K_{oc} (soil adsorption coefficients) which are low, thus giving the pesticide a tendency to leach instead of remaining adsorbed on the particulate matter. The losses of atrazine after application have been estimated to be comparable to other world rivers, with a value of ca. 0.4% of the load.

A number of approaches can be used to estimate fluvial loadings of contaminants to the sea. The approaches used to estimate fluvial loads of atrazine from the Ebro river to the Mediterranean sea [104] were averaging estimators, ratio estimators, and regression-based estimators.

Collective mode (method 1):

$$L = \sum_{i=1}^{12} C_i Q_i$$

where C_i is the pesticide concentration in the month i and Q_i is the total water amount which outflows in the month i .

Averaging method (method 2):

$$L = \frac{365}{12} \sum_{i=1}^{12} C_i Q_i$$

where C_i is the concentration on the i th day and Q_i is the discharge on the i th day.

Ratio method (method 3):

$$L = \left[\frac{\bar{l}}{\bar{q}} \right] Q$$

where \bar{l} is the average daily load, \bar{q} is the average daily discharge and Q is the total flow.

Regression method (method 4):

$$L = \sum_{n=1}^{365} q_n \exp[B_0 + B_1 \ln(q_n)]$$

where q_n is the daily discharge, B_0 and B_1 are the regression coefficients from

$$\ln(c) = B_0 + B_1 \ln(q)$$

where c is the concentration and q is the discharge.

In order to apply such expressions, it is necessary to know the discharge for the Ebro river during a period of at least 1 year. Figure 1.13 shows the discharge ($\text{m}^3 \text{s}^{-1}$) for the Ebro river in the extended Julian day period, 1 June 1994 to 30 May 1995. Table 1.15 presents the annual usage and loads of various pesticides, applying the four methods mentioned above. The four methods can be seen to give very similar results.

The annual usages of the pesticides in the Ebro river basin, 130, 13, 58 and 37 tons/year, respectively, of atrazine, simazine, alachlor and metolachlor [93], make it possible to calculate ratios of loss/usage of these pesticides as 0.63, 3.85, 0.17 and 0.54%, respectively. However, it is necessary to keep in mind the fact that de-ethylatrazine is a direct metabolite from atrazine; therefore, if the two values are summed, the ratio of loss/usage of 0.97% is obtained (the recovery of de-ethylatrazine, 76%, was not applied). The atrazine ratio is within the expected range.

If we consider the usage of the herbicides studied above we can observe a parallelism between the pesticide detection, and the loads and usage for atrazine and metolachlor. In addition, the loads varied between 0.54 and 0.97% and are similar to values observed in other areas for alachlor, of which much more is applied than metolachlor, but only approximately half as much as atrazine, the pesticide loss is only 0.17%, which is much less than for all the other pesticides. This also occurred in Chesapeake Bay and can be attributed to the much larger Henry's Law constant of this pesticide as compared to the other herbicides. In this respect, more air-soil volatilization of alachlor occurs compared to the other major organonitrogen herbicides. Along with its degradative process, this is responsible for the smaller relative percentage of the application mass balance of alachlor which occurs in runoff. The last comment also refers to the herbicide simazine which from the equations for estimating the loads/usage gives a value of 3.85%. This unusually high value for simazine can be attributed to its non-agricultural use in Spain [1]. It is the herbicide used in large amounts in Spain for weed control on railroads, roads, industrial areas, and paths, in amounts of 60 tons per year, whereas the other herbicides studied are not much used for this purpose. This can explain the fact that in the calculation of the ratio of loss/usage, not all the simazine used in that area was considered since only its agricultural use was counted. The non-agricultural usage in that particular area is very difficult to estimate.

Pre-emergent herbicides and their metabolites, particularly atrazine, de-ethylatrazine, metolachlor and alachlor, have been the subject of many studies in the Midwestern US [9,101,105]. In the spring, after the application of herbicides, the concentrations of atrazine, alachlor and simazine were frequently 3–10 times greater than the US EPA maximum contaminant levels. The two major degradation products of atrazine, de-ethylatrazine and de-isopropylatrazine, are usually found in many of

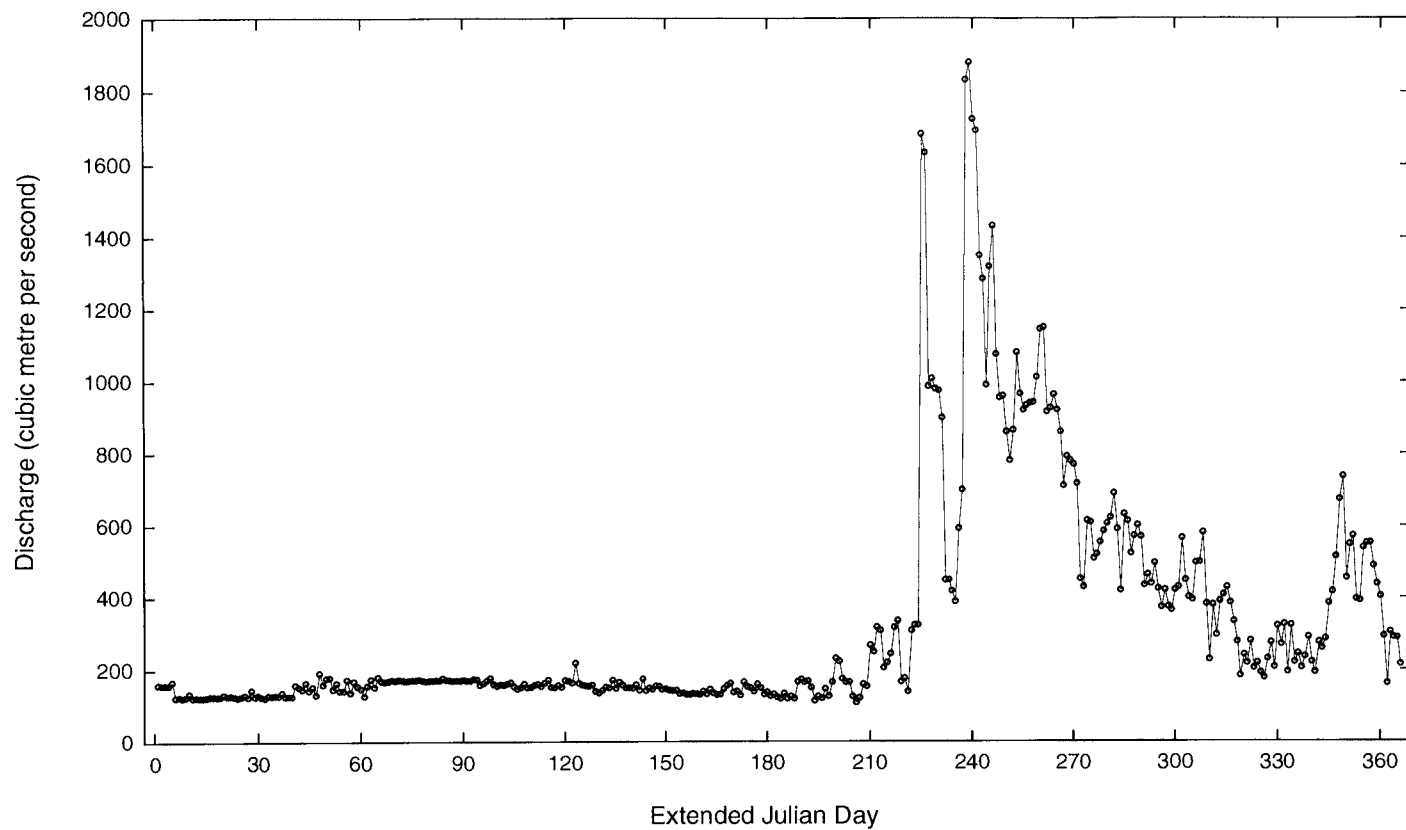


Fig. 1.13. Discharge (m^3/s) for the Ebro river in the extended Julian day period 1 June 1994 to 30 May 1995.

TABLE 1.15
ANNUAL USAGE (TONNES) AND LOADS (KG) OF ORGANONITROGEN PESTICIDES
APPLYING FOUR METHODS OF ESTIMATING FLUVIAL LOADS

Compound	Annual usage (tonnes)	Method 1 (kg)	Method 2 (kg)	Method 3 (kg)	Method 4 (kg)
Atrazine	130	970	817	819	666
Simazine	13	485	503	504	435
de-ethylatrazine	—	496	414	415	337
Alachlor	58	112	92	92	51
Metolachlor	37	236	194	194	144

the monitoring programmes, not only in the US but also in Europe. The order of persistence of the herbicides in surface water is usually atrazine > de-ethylatrazine > metolachlor > alachlor > de-isopropylatrazine > cyanazine. It is interesting to note that de-ethylatrazine occurs more frequently than metolachlor in any pre-planting, post-planting and harvest samples. These findings, which are significant, suggest that some of the parent herbicides persist from year to year in the soil and water samples, that degradation products such as de-ethylatrazine are persistent and mobile and that the ratio of de-ethylatrazine to atrazine can be used to trace ground water movement into rivers. In this respect, a ratio of 0.3 in pre-planting versus a ratio of <0.1 in post-planting sampling was postulated. This large reduction indicates that the runoff of freshly applied herbicides produces a low ratio of degradation product while the harvest sampling, with a ratio of 0.4, may reflect alluvial ground water concentrations of herbicides. It is also accepted that contaminated surface waters may also enter alluvial aquifers by recharge from flood waters and upland runoff [106]. Other pesticides have been identified in current monitoring programmes, in addition to the ubiquitous herbicides described above. Thus, metribuzin, prometon, prometryn, diazinon, molinate, norflurazon and benthocarb have been identified in the Mississippi river and its tributaries, following their use on rice and cotton [85]. Many of the herbicides mentioned above including also bentazone, MCPA, 2,4-D and trifluralin were also detected in Europe in a number of rivers such the Ebro and Rhone and Greek rivers [93,107–110].

A further step in the investigational process is to determine the concentration of herbicides in estuarine and coastal areas at different salinity values. The chlorotriazines have been studied in this respect. For the case of the Chesapeake Bay, which was receiving a significant loading of herbicides from a number of rivers, it was concluded that these materials do not appear to be transported out from the estuary in to the ocean. Nor are they accumulating in bay sediments. Degradation studies indicate that the bay sediments can degrade these contaminants easily, and more rapidly than the inland agricultural environments [111]. The variation in the concentration of atrazine according to the salinity was studied in other experiments performed in

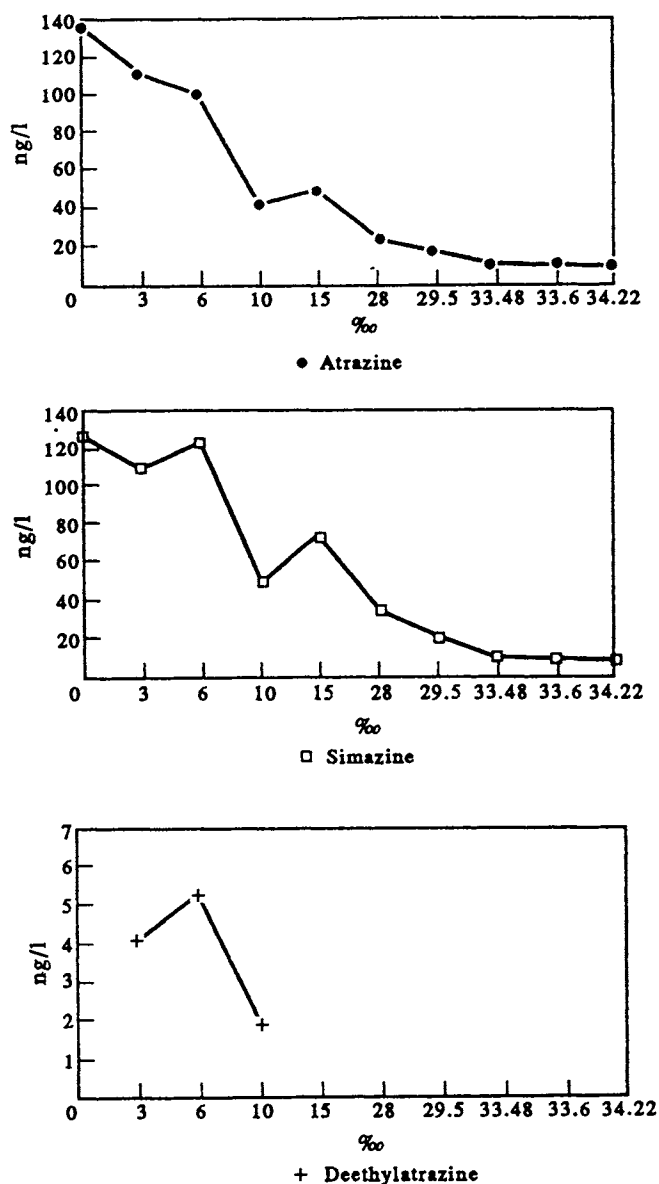


Fig. 1.14. Salinity versus concentration of triazines in estuarine waters.

coastal waters [112]. Sea water samples of different salinity levels, from 3 to 28‰ salinity, were analyzed. Figure 1.14 shows the concentrations of a number of herbicides versus the difference in salinity of the estuarine waters.

In principle, if there were no significant losses in the water column, relatively herbicide-free ocean waters would be expected to mix conservatively with fresh wa-

ter, producing a steady decline of the concentrations of different herbicide as they progress from low to high salinity values. This behaviour agrees with that reported for atrazine in Chesapeake Bay [111,113]. The values found for de-ethylatrazine are very low in all cases, from too-small-to-determine to 23 ng/l. This indicates that the contamination through the estuarine waters, in this particular case, was probably via surface water. For ground water contamination, the levels of de-ethylatrazine would probably be greater because of the enhanced degradation of triazine in soil samples by microorganisms and because of the high solubility of de-ethylatrazine in soil-pore water and, consequently, in the ground water. Atrazine, simazine and terbutylazine were detected in the Baltic and North Sea at levels varying from 1 ng/l up to 1100 ng/l, indicating that the Elbe estuary must be regarded as an area contaminated by triazines [114].

Although the contamination of surface coastal waters by herbicides has been less studied, a new triazine herbicide, Irgarol 1051, has been found at a number of locations on the Côte D'Azur coastline, and in Swiss lake and UK coastal waters [115–118]. This compound inhibits the growth of algae on boats and is incorporated into some marine paints. The data demonstrated the extent of contamination in the French region by leaching of the compound from pleasure boats. Substantial concentrations up to 1.7 µg/l were found in samples from yacht marinas and there was evidence of dispersion of the herbicide into adjacent coastal areas.

Evidence that significant concentrations of herbicides persist in marine systems has been reported. The areas studied were the Ebro delta on the Eastern Coast of Spain, the Rhône delta in the south of France, the river Po in Italy, the Northern Adriatic Sea, and the Termaikos and Amvraikos Gulfs in Greece. These are shown in Fig. 1.15. The most commonly encountered herbicides were atrazine, simazine, alachlor, metolachlor and molinate. In general, the aqueous concentrations encountered in the riverine inputs were comparable to those reported in other estuarine regions of the world. The concentrations generally declined from fresh water locations, through estuaries, to marine waters. Figure 1.16 indicates the concentrations of some pesticides. This pilot survey provided extensive evidence that significant concentrations of some herbicides can persist through fresh water and estuarine environments, to contaminate marine systems [119].

1.3.3.2. Fenitrothion in estuarine waters

In contrast to the relatively high persistence of the herbicides mentioned above, organophosphorus pesticides are degraded rapidly in estuarine coastal areas [55,71,120,121]. Fenitrothion is used in the Ebro delta for the elimination of rice-stem borers and was applied to areas within the irrigation catchment of the Ebro delta with two estimated concentrations, of 200 and 20 µg/l. The evolution of the level of fenitrothion and the formation of its transformation products, 3-methyl-4-nitrophenol, fenitrooxon and the *S*-methyl isomer of fenitrothion, was recorded during 4 days after application. The concentration of fenitrothion decayed sharply

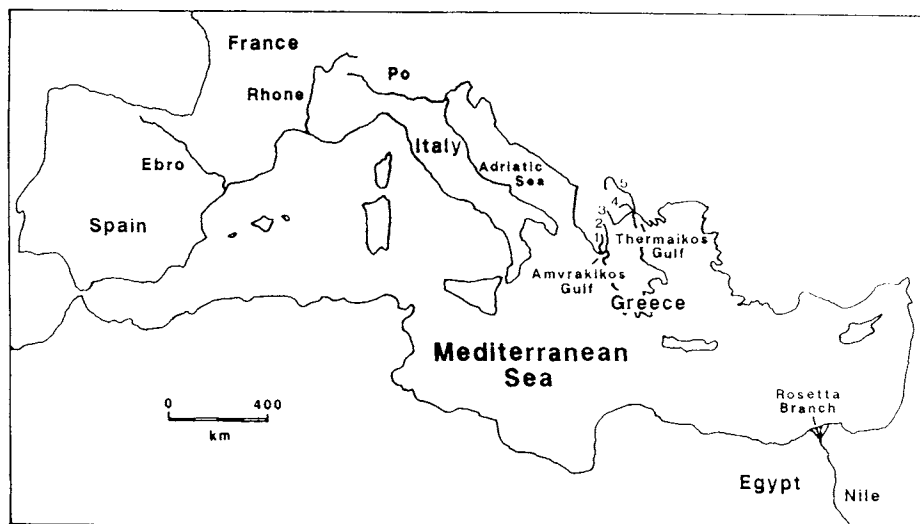


Fig. 1.15. Mediterranean estuaries investigated by the herbicide pilot survey. The rivers in Greece are identified by the numbers corresponding to: 1, Louros; 2, Arachthos; 3, Aliakmon; 4, Loudias and 5, Axios.

within 2 h, to 6.0% and 0.3% of the initial concentration applied, and reached a steady state within 10 h with concentrations of 4 and 0.01 $\mu\text{g/l}$, respectively, which remained constant during the 4 days that the experiment lasted. The transformation products were encountered at a very low concentration, around 0.01 $\mu\text{g/l}$.

The chemical degradation can be described using a first-order degradation curve:

$$C_t = C_0 \exp(-kt)$$

C_t is the concentration of the pollutant at time t , C_0 is the initial concentration, k is the rate constant (slope).

Then the half-life time, $t_{1/2}$, is $\ln 2/k$. By plotting the natural logarithm versus time (hours after treatment), a straight line can be obtained, and the rate constant, k derived from it.

The half-life of fenitrothion is estimated to be 13 h, with a disappearance rate of 0.053 $\mu\text{g l}^{-1} \text{h}^{-1}$. The evolution of the levels of fenitrothion under the natural environmental conditions of application is shown in Fig. 1.17.

Figure 1.18 shows the degradation pathway for fenitrothion under these conditions. In this case, it is clear that there is no problem in the coastal estuarine waters since rapid degradation of the compounds occurs within the first few hours after application. This contrasts with the behaviour of triazines, where a permanent contamination exists in the coastal areas receiving surface waters polluted with triazine herbicides.

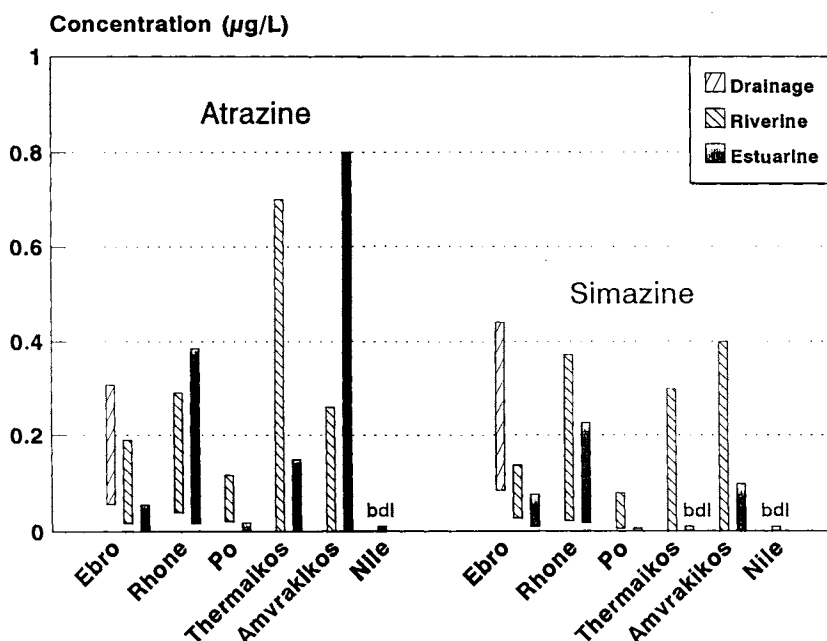


Fig. 1.16. Ranges of aqueous concentrations of atrazine and simazine in the selected Mediterranean locations.

In a more recent study [121], the behaviour of aerially applied fenitrothion was studied. For checking the actual amount of the commercial product, Tionfos, that was applied, ELISA was implemented for both the commercial product (50% of fenitrothion) and the diluted product (3.7% of fenitrothion). The ELISA results for Tionfos and the diluted product were 48 and 3.5%, respectively, indicating that ELISA is without interference at these concentrations and that the determination of formulated product was correct. Twelve samples were also measured with ELISA alone, after filtration and acidification immediately after collection, and also by Prospekt LC-DAD after C_{18} extraction disks. The samples were stored at -20°C for 2 months. A high correlation was obtained between the two techniques ($r = 0.999$) when the data before the extraction with C_{18} disks were compared.

Figure 1.19 shows the decay of fenitrothion with time, measured by ELISA. Fenitrothion was plotted in the form of the natural logarithm of the concentration versus time. The highest results were obtained by Prospekt LC-DAD. Moreover, between 5 and 10 h, a peak was detected by the two analytical techniques. The rate constant, k , and half-life times $t_{1/2}$ were calculated from linear regression curves; the correlation coefficients were about 0.9, and consequently the k and $t_{1/2}$ values can be considered as representative values. From these two curves, the disappearance rate or rate constant, k , was calculated as 0.036 for ELISA and 0.063 for LC-DAD, and the

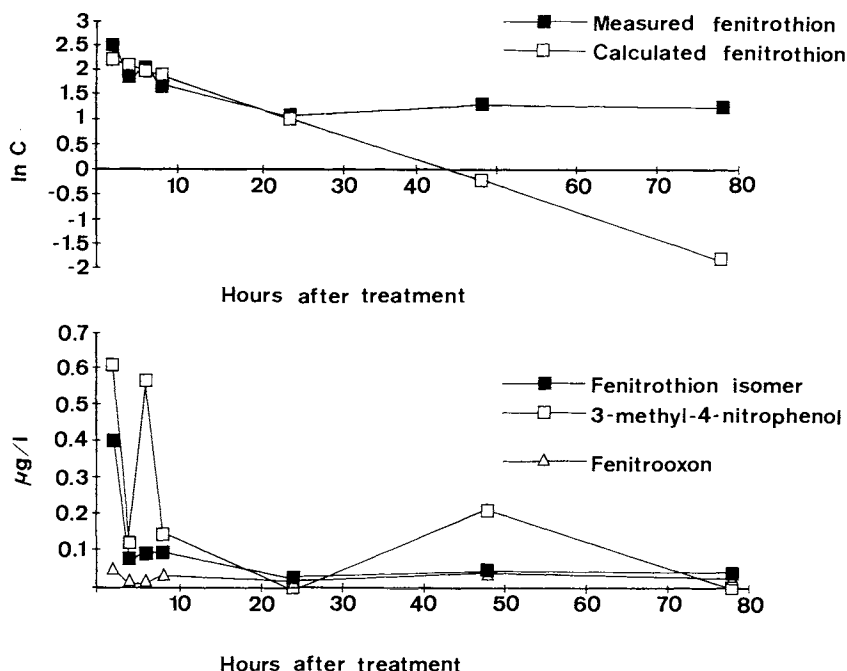


Fig. 1.17. Evolution of the concentration levels of fenitrothion and its transformation products after the application of fenitrothion at a concentration of 200 $\mu\text{g/l}$.

half-life times $t_{1/2}$ were 19.3 and 11 h, respectively, after treatment. The initial concentration of fenitrothion was monitored directly by ELISA in the rice-crop field waters as 119 $\mu\text{g/l}$ at zero time; this value has not been plotted in Fig. 1.19.

Table 1.16 shows the results for half-lives reported by other authors. Lartiges and Garrigues [122] obtained a half-life of 3 days in experiments carried out under sunlight. Other field experiments in a lake gave half-lives between 0.9 and 7 days [123]. For the same Ebro delta ditch area during the winter period, the half-life was 13 h [71]. Mikami et al. [124] found that the rate and degradation pathways for fenitrothion by photolysis in natural river- and sea water were similar to those in buffered solutions having the same pH. The photolysis rate was not significantly affected by the pH (3–9) or the compound concentrations (1 or 10 mg/l), with half-life values below 1.5 days.

The increase in temperature plays a role in the degradation of fenitrothion, with a reduction in half-life from 49.5 to 4.7 days when the temperature was raised from 23 to 49.7°C [124]. Photolysis has even been reported to be the major degradation process [122,124,125]. At higher temperatures and with sun irradiation, higher degradation is expected and it explains most of the results reported in Table 1.16, which are in agreement with the relatively high quantum yield of fenitrothion [126].

Volatilization has already been reported to be a major source of pesticide loss in

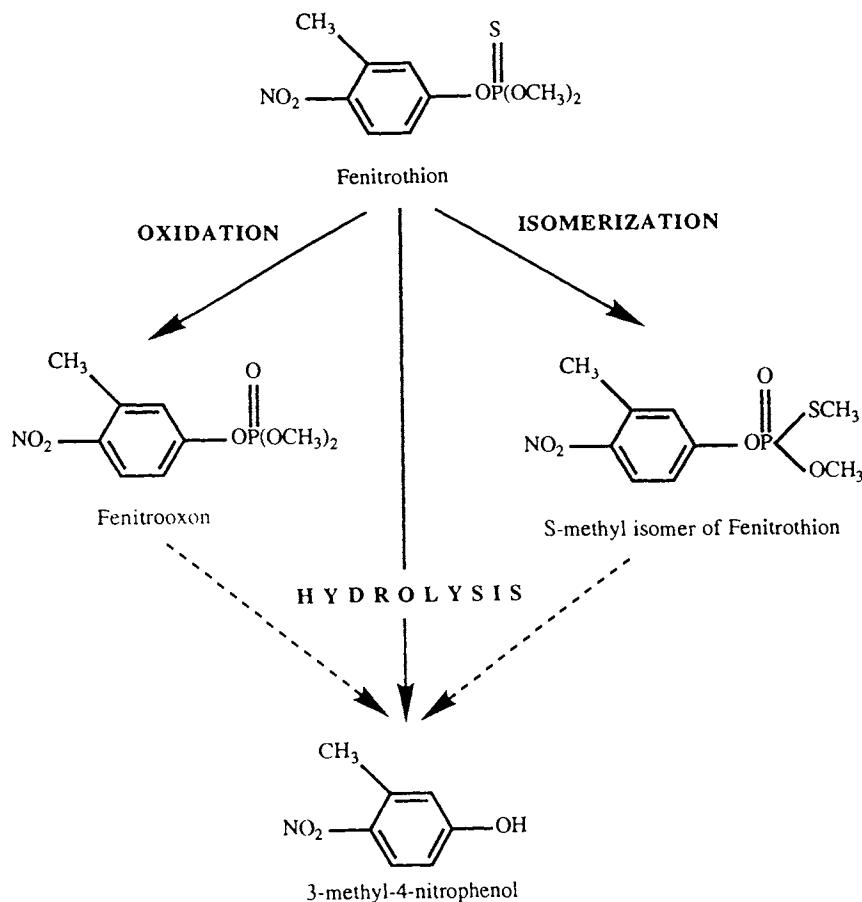


Fig. 1.18. Decomposition of fenitrothion in estuarine waters.

the experiments performed in a Canadian lake [123]. It can be explained by Henry's Law constant (HLC), which has a value of 0.0036 Pa m³/mol calculated at 20°C [41]. The HLC is generally calculated from the vapour pressure and water solubility. Considering the increase in temperature where the experiments were performed, it will have not much influence on the water solubility of fenitrothion (30 mg/l), but the vapour pressure increases at higher temperatures and, consequently, the HLC will be somewhat higher.

It is likely, therefore, that photolysis and volatilization from water should be considered to be major sources of fenitrothion loss after aerial application in the Ebro ditch water during the summer period.

The last comment to be made on the fate of pesticides in estuarine areas is that very few studies are currently available on monitoring data and the fate of pesticides in tropical countries. Readman et al. [127] determined a few organophosphorus pes-

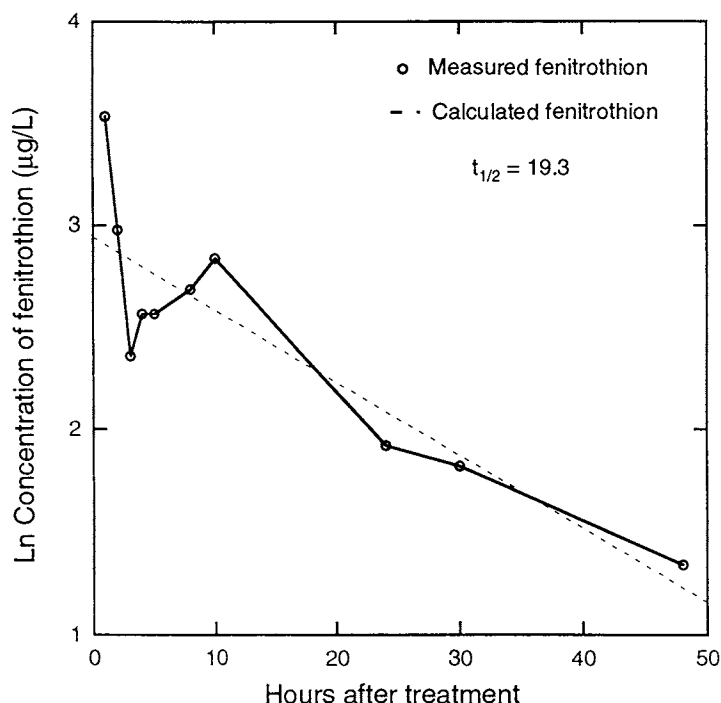


Fig. 1.19. Evolution of the concentration levels of fenitrothion measured by ELISA after filtration (plotted as ln of concentration versus time, giving both measured values and calculated values, according to a first-order rate equation).

TABLE 1.16
COMPARATIVE DATA OF HALF-LIVES FOR FENITROTHION UNDER VARIOUS WATER
TYPES AND ENVIRONMENTAL CONDITIONS

Half-life (h)	Water			
	pH	Temperature (°C)	Type and location	Conditions
72	8.1	22–25	Sea water (Arcachon Bay, France)	Dark
15–168	6.7	11 (summer)	Palfrey lake (Canada)	Sunlight
13	7.8	11 (winter)	Irrigation ditch from Ebro Delta (Spain)	Sunlight
11–19.3	7.8–8.2	25–30 (summer)	Rice crop field from Ebro Delta (Spain)	Sunlight
70–74	8.2	15–18	Ebro Delta (Spain)	Dark
36–48	7.0–7.5	19–23	Lac Bourgeois (Quebec)	Sunlight
518.4–1188	7.5	23	Lac Bourgeois (Quebec)	Dark
26.4	7.4	25	Muko river (Takarazuka, Hyogo, Japan)	Sunlight

ticides in tropical marine environments and, recently, Castillo et al. [128] reported an overview of pilot surveys of pesticides in tropical ecosystems of Central America. Although many studies are available regarding DDT and organochlorinated pesticides in the developed countries, very few studies can be found on the use of polar pesticides in tropical environments. Studies such as those reported on Ebro delta water [55,71,120,121] may give approximations to the behaviour in tropical countries. As mentioned earlier, it is desirable that developed countries that are in the list of top consumers of pesticides for agricultural use should undertake fate and risk-assessment studies in order to give a better understanding of the behaviour of polar pesticides in their rivers and estuaries.

1.3.4. Pesticides in other waters (rain, storm, lakes, etc.)

Lakes are transient reservoirs for many of the pesticides and reflect the quality of the surrounding environment. Their composition and properties are less affected by temporal fluctuations than those of rivers and streams. Rain and snow samples are also analyzed for pesticides. In Switzerland, it was shown that volatilization, and wind erosion of soils from areas treated with triazine herbicides contaminated some lakes in remote areas by aerial transport and subsequent deposition. Other major inputs of pesticides in lakes were also attributed to their non-agricultural application along roads and railroads [129]. Other pesticides, such as atrazine, isoproturon, lindane and terbutylazine were in precipitation collected in bulk samples and in wet only samples in Northern Germany. These pesticides were found in more than 20% of the rain samples analyzed [130]. The fate of the pesticides could not be explained by their vapour pressures alone, which would determine their volatility and the washout from the atmosphere. It was concluded that the amount of pesticides in rain water is also decisively influenced by the extent of their application, which may explain the presence in rain water of, e.g., isoproturon which has a relatively low vapour pressure. Once a pesticide is present in the atmosphere, transformation processes such as photolysis can occur. This is the case for pesticides with relatively high photochemical reactivity, such as chlorpyrifos, pirimicarb, vinclozolin and propoxur. The maximum concentration levels found for pesticides in rain in Germany were $1.1 \mu\text{g/l}$. It was concluded that studies of the occurrence of pesticides in rain water must also take into consideration the amount of other contaminants, e.g., PAHs which are emitted by the burning of fossil fuels. In this respect, pesticides contribute to only a minor extent to the organic load of rain [130]. Ten pesticides (alachlor, atrazine, cayanazine, EPTC, fonofos, metolachlor, propachlor, metribuzin, simazine and trifluralin) were analyzed from lakes of Northern Nebraska. Their maximum impacts were associated with a flush of pesticides in the months of May and June when runoff pesticide concentrations are quite elevated. A relatively short lacustrine half-life for atrazine was associated with the suspended solids in these lakes and so surface-catalyzed hydrolysis was assumed to be responsible for such mechanisms.

The half-lives in the lakes were estimated using pseudo-first-order kinetics and were considerably longer than the published half-lives. For atrazine, alachlor and cyanazine the estimates were 193–124 days, 21 and 103 days, whereas the literature data for soil-half-lives are 60, 15 and 14 days, respectively. The longer estimated lake half-lives reflect cold winter water temperatures and generally less favourable degradation [131].

An interesting study, reports data on the pesticide concentrations transported from its river tributaries to lake Erie [132]. The Erie basin has land uses ranging from 80% agricultural to mostly urban and afforested and the concentrations of major pesticides there, between 1983 and 1991, varied from levels below $1\text{ }\mu\text{g/l}$ up to almost $100\text{ }\mu\text{g/l}$. Atrazine, alachlor, metolachlor, metribuzin, cyanazine and linuron were then the most detected pesticides. Other pesticides detected, at much lower levels, were terbufos, butylate, chlorpyrifos, EPTC, phorate, fonofos and simazine. On an annual basis, elevated monthly average concentrations were usually observed from May to August and low concentrations were present for the rest of the year. The average concentrations in tributaries were correlated with the amounts applied in the basin but with important secondary effects from the compounds chemical properties and modes of application. During runoff or storms following application, the concentrations rise rapidly, about the time peak discharge and decline slowly afterwards. There are further studies of pesticides in water matrices, especially for US and Canadian lakes [123]. The behaviour of pesticides in large lakes follows the patterns for surface waters, in general.

During the last few years increasing concern has been devoted to toxic air contaminants. In the US, the Department of Pesticide regulation is responsible for the development of a list of candidate pesticides to be investigated as toxic air contaminants. This list is to be updated as needed and eventually all pesticides used agriculturally will be scrutinized. A pesticide is chosen through a series of considerations that take into account potential adverse health effects, the amount, manner and region of usage, and the physical and chemical properties of the compounds under consideration. A Candidate Pesticide Toxic Air Contaminants List was proposed in California and is given in Table 1.17 [133].

Since the purpose of this book is to discuss the analysis of pesticides in the aquatic environment, we shall not discuss further the problem of pesticides in rain, or the atmosphere in general. However, we should indicate that concern exists about these chemicals in the air, and that this is an area where research will probably grow in the near future.

1.3.5. Pesticides in aquatic organisms

Some physico-chemical properties of pesticides are relevant to their bioaccumulation. These include their water- and fat-solubility, K_{ow} , their adsorptivity onto soil/sediments, lipids and proteins, their molecular mass, hydrogen-bond formation,

TABLE 1.17
CANDIDATE PESTICIDE TOXIC AIR CONTAMINANTS IN CALIFORNIA

Ethyl parathion	Mancozeb
Methyl parathion	1,3-Dichloropropene
Paraquat	Ziram
Aazinphos-methyl	Naled
Bromoxynil	Carbofuran
Benomyl	Oxydemeton-methyl
Chlorothalonil	Triadimefon
Captan	Methidathion
Chloropicrin	Acephate
Methomyl	Permetrin
Methyl bromide	Diquat bromide
Terbufos	2,4-D

dissociation constants, vapour pressure, and melting points. The bio-accumulation properties of many pesticides have already been indicated in Table 1.10.

As a rule, the bio-accumulation of pesticides in aquatic organisms is related to their *n*-octanol–water partition coefficient (K_{ow}). Also, the bioaccumulation factors (BCF) exhibit a linear relationship to $\log K_{ow}$, but only up to a value of $\log K_{ow}$ of 5–6. Alternative correlations including quadratic terms to account for steric factors, and metabolism terms have also been reported. Such additional factors are considered to be essential for use with compounds such as PCBs, which have $\log K_{ow}$ values greater than 5 or 6 [134].

Modern pesticides have rather different $\log K_{ow}$ values, depending on their structural family. Thus, $\log K_{ow}$ for triazines varies between 1.95 and 3.38, which suggests a low bio-accumulation potential, while organophosphates and carbamates have $\log K_{ow}$ values in a range as wide as 0.7 to 5.9 [31,135]. The highest values (above 5) correspond to organophosphorus pesticides such as bromofos-ethyl, chlorpyrifos, ethion, iodofenphos, leptophos, temephos and trichloronat, whereas the lowest (<2) are usually those of carbamates. Very few studies have been conducted on the bioaccumulations of modern pesticides into biota (and are not within the scope of this book). Most of the studies carried out on the bioaccumulation of pesticides in biota relates to organochlorinated pesticides. Two studies [55,136] report the bioaccumulation of organophosphorus pesticides in biota samples. From these two studies it is worthwhile mentioning the high bioaccumulation of fenitrothion in the fish *Gambusia affinis* which reached values up to 300 ng/g wet weight, whereas for the other organophosphorus pesticides, the mean concentrations were below 40 ng/g. The bio-accumulation of fenitrothion was related to its use, since it is applied aerially and is the compound used in large amounts (40–50 tons per year) in the area of the Ebro estuary [121,136]. In a recent study carried out in Central America [128], it was reported that pesticides such as 2,4-D and 2,4,5-T were detected in coral tissues and organophosphorus pesticides such as parathion and paraoxon in fish and mussels;

chlorpyrifos was detected in marine biota samples from various Central American countries, with levels up to 4 mg /kg. However, most of the bio-accumulation values reported in the same study were for organochlorinated pesticides, which are not the subject of this book. Nevertheless, they are usually found in biota samples and many environmental studies are being conducted on their bio-accumulation.

1.4. DEGRADATION OF PESTICIDES IN THE AQUATIC ENVIRONMENT

1.4.1. Laboratory experiments

After application, pesticides are transformed, mainly by biotic and abiotic processes. Photolysis and hydrolysis are the two main abiotic processes that take place in aquatic systems. In photolysis, the light energy and intensity, and the duration of sunshine, direct or indirect, affect the rates of degradation via the formation of active species. The influence of many environmental parameters, such as the pH and type of water and of humic substances, which can catalyze or retard the hydrolysis or photolysis, are poorly understood. Several reports indicate that humic substances can either catalyze or retard the hydrolysis or photolysis of pesticides.

Many degradation studies are performed under laboratory conditions in order to simulate real, natural environmental situations. In such studies it is of interest to investigate the effects of sunlight under natural conditions. We need to consider the facts that the wavelength distribution varies diurnally and seasonally and also that the intensity of sunlight varies during the day. It is also known that clouds cause fluctuations in the intensity. In normal conditions the concentration of the pollutants is in a very low range which does not prevent light penetration through the entire depth of the solution (this is better than higher concentrations of pollutants) [137]. Other experiments, using dark samples, and different types of water, such as sterilized and non-sterilized, drinking, well, surface, and estuarine water, should be performed in order to assess the behaviour of chemicals under real conditions. The use of sterile water and samples kept in the dark are also necessary so that one may compare the data and establish the various mechanisms, whether of hydrolysis, photolysis or microbial degradation, which are effecting the degradation of the pesticides in water.

For experimental equipment, Pyrex glass is recommended and generally employed despite the fact that it absorbs light at some wavelengths and, in principle, it is more desirable to use quartz glass. It is accepted that when Pyrex borosilicate glass vessels and Teflon are used they transmit less than 1% of the radiation at <290 nm, but they are usually preferred for such studies [138] because they are much more economical than quartz.

Photolysis is one of the major transformation processes affecting the fate of pesticides in the aquatic environment. In this context, three main light sources are used

for carrying out model photolysis experiments, i.e., natural summer sunlight, suntest apparatus, and mercury lamps. It has been demonstrated that the use of different light sources under identical aqueous conditions can produce similar degradation products, with the only difference being in their kinetics of formation [139,140]. Although the use of xenon arc lamp with light above >290 nm is preferred, since the results obtained can be compared more easily to real sunlight conditions, mercury lamps, with an intensity-maximum close to 254 nm have been used for convenience and when the photodegradation of the compounds is otherwise too slow. The xenon arc lamp has been shown to be equivalent to natural sunlight for aqueous photolysis studies of several compounds, such as carbaryl. The wavelength range varies from 300 to 800 nm, which is a radiation very close to natural sunlight [141]. An example of the radiation obtained from the xenon arc lamp, compared to natural sunlight, is shown in Fig. 1.20 [140].

In some studies, photosensitizers are used intentionally to enhance the degradation of pesticides in water. This is the case with acetone, a known triplet sensitizer that occurs in almost every natural aquatic environment, or methanol, which is used to dissolve pesticides in water. Surfactants which are usually found in pesticide formulations can also affect the photolysis of pesticides. They usually cause an increase in the photodegradation rate of herbicides having relatively low water solubilities, chloro substituents on their aromatic ring, or triplet energies below that of the surfactant. Many examples of the environmental photochemistry of herbicides have been reported: one is the excellent review paper by Marcheterre et al. [142]. In this introduction we discuss some examples of the photodegradation of pesticides in water.

The photolysis of chlorotriazine, organophosphorus, and phenylurea pesticides has been studied in a variety of aquatic media under laboratory conditions. The experiments have revealed differences in degradation rates between natural (whether surface or sea waters) and distilled waters. These arise from the presence of particulate and dissolved substances in natural waters which influence photolysis by fostering radical reactions and hence increase degradation rates. Photosensitizing and quenching effects have been observed for a variety of pesticides (atrazine, diuron) in photodegradation experiments involving distilled water or sea water [143,144].

A study of the aquatic photodegradation of pesticides from four different groups such as the carbamates, chlorotriazines, organophosphorus compounds and phenylureas (aldicarb, carbaryl, cyanazine, fenitrothion, linuron, and parathion-methyl) has been published by Durand et al. [145]. The irradiated samples were analyzed by thermospray LC-MS in order to determine as many as possible of the metabolites. In general, when other types of water, e.g., lake and sea water, are employed, quenching effects are observed for carbaryl which thus retard its photodegradation relative to other pesticides.

Photodegradation studies under laboratory conditions have been carried out for a variety of pesticides. That of atrazine, a chlorotriazine, showed that hydroxyatrazine was one of the main degradation products formed in various water samples. By using

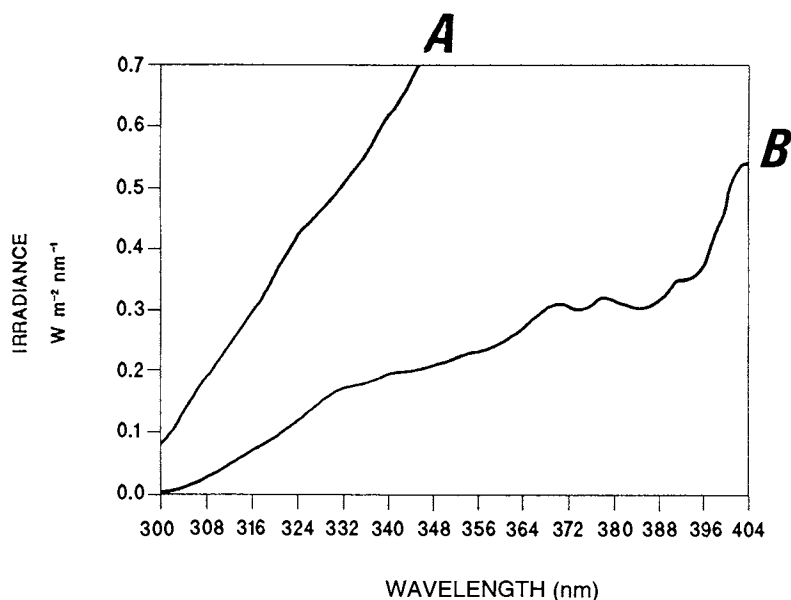


Fig. 1.20. Comparison of spectral radiation between 286 and 400 nm of (A) xenon arc lamp and (B) Almeria sunlight.

LC/TSP-MS/MS for the identification of aquatic photoproducts from chlorotriazine pesticides, obtained after UV-Suntest irradiation which simulates real sunlight conditions, the formation of hydroxy-, methoxy-, hydrogenated-, and dealkylated derivatives was unequivocally detected. By using the loss of the neutral fragment of mass 42 it was possible to achieve high selectivity in the determination of the triazine metabolites at low LODs. The MS/MS analysis of the photodegraded solutions of de-ethylatrazine used the “42 neutral loss” and selective detection of H-de-ethylatrazine (HDEA), hydroxy-de-ethylatrazine (HYDEA), methoxy-de-ethylatrazine (MDEA), and de-ethylatrazine (DEA) was obtained [146].

1.4.1.1. Alachlor photodegradation

An extensive degradation study was carried out for alachlor in water, by irradiating it with a xenon arc lamp [147]. The UV spectrum and structure of alachlor are given in Fig. 1.21. It absorbs radiation weakly between 290 nm, which is the lower wavelength limit for sunlight, and 320 nm. The direct photolysis of alachlor is therefore slow, and when analytical grade material was irradiated in distilled water, it was all destroyed within 10 h. Alachlor is fairly stable in river, water and no loss by chemical hydrolysis processes was found after at least 1 month for solutions kept in the dark at pH 6.8 (ammonium acetate buffer). In the photolysis, the appearance of

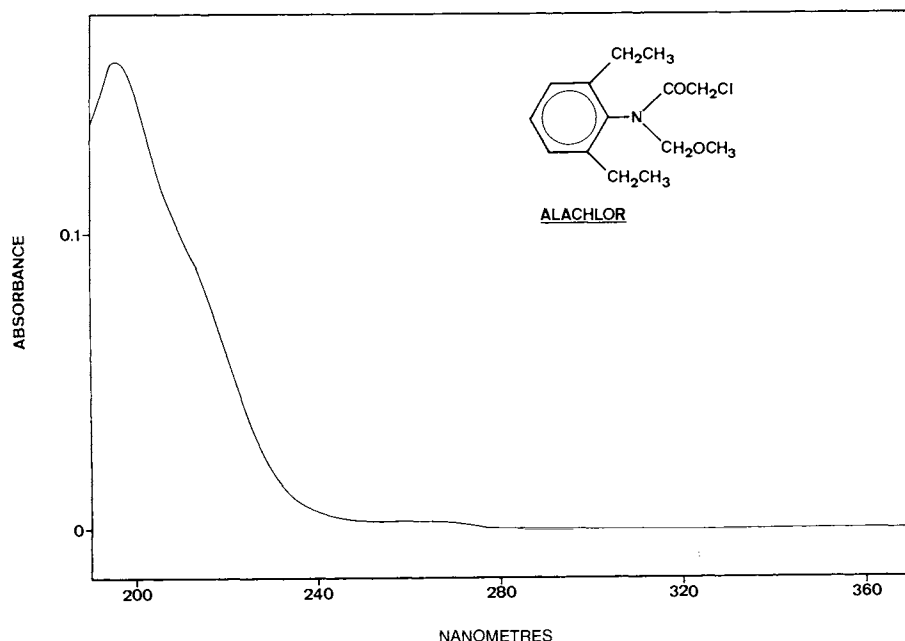


Fig. 1.21. Chemical structure and UV/vis spectra of alachlor .

hydroxy-alachlor followed first-order kinetics, suggesting that a stable radical intermediate is formed which reacts with water. The formation of the intermediate can be explained by the loss of chlorine, following the absorption of photons; its structure is given in Fig. 1.22. Such a radical is probably the origin of a complicated photolysis mechanism. We should also point out that alachlor in solution has a conformational equilibrium around the amide bond but this which has no effect on its rate of loss in water.

No differences were observed between the photolyses of analytical grade alachlor and formulated alachlor. When humic substances (HS) were added to the water they were partly bound to alachlor. The binding of alachlor to HS was demonstrated by irradiating alachlor solutions during 15 h in order to ensure that photodegradation of the dissolved alachlor was achieved. However, changing the pH from 7 to 1 leads to release of the pesticide. This alachlor fraction was bound to HS and was never available to photolysis action. The binding fraction was estimated to be about 5% of the initial alachlor concentration. Binding by physical sorption is reversible and involves Van der Waals forces, rather than covalent binding in stable aggregates. The masking behaviour of humic matter was optimum within a contact time of 24 h before irradiation. At a constant pH 6.8 an increase in ionic strength (0.05–0.1 M of NaCl) induced a reduction in binding rate. Therefore the K_{oc} of alachlor depends on its solubility in different waters. Furthermore, different HS were extracted from Rhône

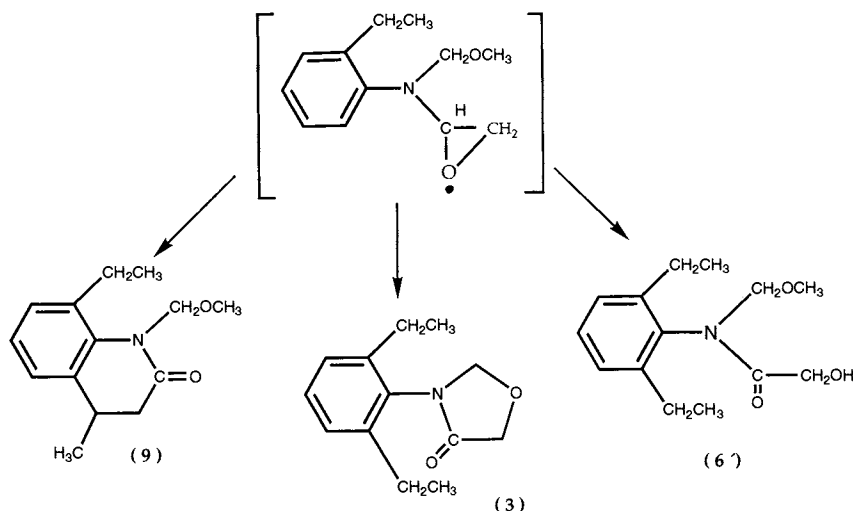


Fig. 1.22. Chemical structures of the photochemical products of alachlor: (6'), hydroxyalachlor; (9), 8-ethyl-1-methoxymethyl-4-methyl-2-oxo-1,2,3,4-tetrahydroquinoline and (3), [4-(2,6-diethylphenyl)-3-morpholinone].

river-delta water samples with a water salinity rate of 0, 4.5, 6.2, and 33%, respectively, and their binding behaviours investigated. The binding fraction of alachlor increases when the water salinity rate increases, reaching 10% for HS extracted from water with 33% salinity. The binding of pesticides by HS may depend not only on the chemical characteristics of the water but also on the structural characteristics of the HS. Fourteen photodegradation products have been detected by using either UV or MS detection and compound molecular weights. After photodegradation, a total of 14 photoproducts was found resulting from the dechlorination of the alachlor, with subsequent hydroxylation and cyclization processes. The two major photoproducts were hydroxyalachlor and 8-ethyl-1-methoxy-methyl-4-methyl-2-oxo-1,2,3,4-tetrahydroquinone [147]. The calculated rate constants and half-lives of alachlor in water under xenon-arc irradiation are indicated in Table 1.18. In this work it was concluded that alachlor is moderately

TABLE 1.18
RATE CONSTANT AND HALF-LIFE OF ALACHLOR IN WATER UNDER XENON ARC LAMP IRRADIATION

Water type	Rate constant ($\text{ng ml}^{-1} \text{ min}^{-1}$)	Half-life (min)
Distilled water + pure alachlor	1.79×10^{-2}	140
Distilled water + formulated alachlor	1.94×10^{-2}	129
Ebro river water + pure alachlor	2.98×10^{-2}	83.8

persistent towards photolysis, and the same transformation products were found in distilled and natural waters. The major point is that the work reported expanded the list of transformation products that could be formed in water samples under different environmental conditions and that some of the compounds formed matched metabolites found in real water samples [98]. The use of appropriate analytical techniques such as like LC-MS facilitates the identification of new photoproducts.

Alachlor, which is one of the top ten herbicides used worldwide [3], is one of the few compounds whose behaviour has been studied by photocatalysis, using also a xenon arc lamp [148]. Photocatalytic oxidation by semiconductor oxides such as TiO_2 , or by Fenton's reagent represents another area of environmental interest but, in this case, for the treatment of contaminated waste waters. In the presence of oxygen and near-UV light many organic pollutants can be rapidly transformed into less toxic compounds. The most commonly used photocatalytic oxidation reaction reported in the literature involves TiO_2 in water and has been used for a variety of chemical pollutants.

Fenton's reagent (Fe^{2+} with H_2O_2) provides a system which has been scarcely used for the elimination of contaminants. Its main advantage over other hydroxy-radical-generating systems is that it is cheaper to use than TiO_2 particles or ozone generators.

A comparative degradation study has been performed of alachlor, spiked at 30–60 $\mu\text{g/l}$ in water, using photocatalysis with FeCl_3 or TiO_2 , and photolysis using a xenon-arc photoreactor. After irradiation the water samples were pre-concentrated, using solid-phase disk extraction with C_{18} disks and analyzed by gas chromatography, electron capture and mass spectrometric detection (GC-ECD and GC-MS, respectively). Three photoproducts unequivocally identified were 2-hydroxy-2,6'-diethyl-*N*-methylacetanilide, 8-ethyl-1-methoxymethyl-4-methyl-2-oxo-1,2,3,4-tetra-

TABLE 1.19

HALF-LIVES (HOURS) OF CARBOFURAN AND METHIOCARB IN TWO WATER TYPES: (A) UNDER XENON ARC LAMP; (B) HALF-LIVES OF THE TOTAL TOXIC COMPOUNDS (METHIOCARB + METHIOCARB SULFOXIDE) UNDER XENON ARC LAMP; (C) UNDER SUNLIGHT IRRADIATION; (D) DATA REPORTED IN REF. [140], USING NATURAL SUNLIGHT IRRADIATION

Water type	Carbofuran				Methiocarb			
	A	<i>k</i>	C	D	A	<i>k</i>	B	C
Distilled water + pure compound	1.3	0.53	360	660	0.31	2.22	0.95	192
River water + pure compound	1.7	0.40	134	173	0.38	1.80	1.0	100
Distilled water + Mesurol ^a					0.60	1.15	1.50	216

n.d., not determined. Calculated rate constant, k (h^{-1}), when using xenon arc lamp. Half lives were estimated using LC-PCR-FD analysis (see experimental part).

^aMesurol is a commercial formulation of methiocarb.

hydroquinoline, and hydroxylalchlor. Two further photoproducts were detected by GC-MS: their chemical structures are unknown but they have base peaks at m/z values 146 and 218, and molecular weights of 221 and 233, respectively.

Although alachlor showed high stability in water when it was exposed to natural sunlight irradiation for a period of more than 120 h, the use of photocatalysis with FeCl_3 or TiO_2 rendered the degradation very fast, with half-lives varying from 10 to 17 min. The degradation followed first-order kinetics and the coefficient of variation, for $n = 4$, varied from 2 to 16 or 27 to 33% under photocatalysis or photolysis. Several of the metabolites identified matched those detected in real matrices [98].

1.4.1.2. Photolysis of carbofuran and methiocarb

Another example of the use of the xenon arc lamp is the irradiation of water samples containing carbofuran and methiocarb [140]. Table 1.19 includes the values of k , the first-order rate constant. The half lives ($t_{1/2}$) are calculated according to the relationship $t_{1/2} = \ln 2/k$. The degradation of carbofuran and methiocarb were fast, both pesticides being depleted within 3 and 5 h, respectively. Both reactions fit pseudo-first-order kinetic forms of plots of $\ln C/C_0$ versus time, with the use of PCR-FD detection for quantitation purposes. Half-lives in distilled water were estimated around 0.31 and 1.3 h, for methiocarb and carbofuran, respectively. When experiments were carried out on river water, a slight increase in the half-life of carbofuran was observed, whereas for methiocarb the effect of dissolved organic matter (DOM) was very small (an increase in half-life from 0.31 to 0.38 h), and considered to be of no significance. Clearly there is rapid photolysis of the compounds. Although HO^\bullet radicals from DOM are present in the solution, their concentration is very low and will have no effect on these rates. Indeed, oxidations by HO^\bullet , $^1\text{O}_2$ and RO_2 are all too slow to compete.

To conclude our discussion of degradation studies of pesticides under laboratory conditions, we should indicate that the use of the xenon arc lamp to simulate the natural degradation of pesticides in the environment is of interest in the context of simulating real environmental conditions. The concentrations of the analytes studied should be at low ppb levels, close to environmental conditions. This is possible because the current analytical techniques have improved limits of detection and make it possible to use concentrations as close as possible to environmental levels. Another point for consideration is that the xenon arc lamp does not fully match real sunlight conditions, and is usually somewhat harder than the natural conditions. Figure 1.20 provides a comparison between the spectral irradiance obtained from the xenon arc lamp (A) and the natural sunlight of Almeria, in the South East of Spain (B). It clearly shows that the xenon arc lamp provides shorter wavelength radiation and can consequently degrade the analytes faster. In any case, the xenon arc lamp still provides the most widely used approach for comparing laboratory conditions with natural sunlight and is an appropriate method to study the transformation of pesticides in the laboratory.

Another way of performing laboratory studies of pesticides is to use an Hg lamp instead of a xenon arc lamp [149]. However, these experiments do not reflect environmental conditions since the high pressure Hg lamp gives radiation of much lower wavelength than the xenon arc lamp and is consequently less closely comparable to environmental conditions. These studies were common a few years ago but they used pesticides at the high ppm level, dissolved in organic solvents, which reflect natural environmental situations even less. The most interesting approach nowadays is to use situations as close as possible to the aquatic environment. This is helped by the recent achievements in analytical techniques that have permitted considerable reductions in the limits of detection.

1.4.2. Field studies

The effects of the photochemistry of pesticides in natural waters have been described in a review [150]. It was concluded in this study that solar radiation is fundamental to all photochemical and photobiological processes in natural waters. Differentiation was made between fresh water and salt water ecosystems, and it was pointed out that the illuminated zones of fresh waters are generally much shallower and more variable than those of central oceanic and pristine lakes, which have up to 1% of the surface light at 100–150 m. The dissolved organic compounds and suspended particles are variable in both environments but are usually one to four orders of magnitude lower in sea water. The pH of sea water is around 7–8 whereas that of fresh water is 7–10, and sea water's ionic strength is 0.7 whereas fresh water's is 0.001–3. Also, the anionic composition of sea water is constant, dominated by chloride and sulfate, whereas that of fresh water varies and is usually dominated by bicarbonates.

In one example, the degradation of various organophosphorus pesticides in natural estuarine waters was reported. It is known for these compounds that the hydrolysis resulting from a change from 6.1–7.4 enhanced the degradation of malathion, methyl-chlorpyrifos, ethyl-chlorpyrifos and parathion [151–153].

The specific objectives of a reported field study were as follows [154]: (1) to evaluate the decay of ten organophosphorus pesticides and one formulation in estuarine waters, when exposed during several weeks to natural day- and night- irradiation under similar temperatures and natural conditions similar to those in a real estuarine area; (2) to monitor the presence and concentrations of various TPs, and to calculate the half-lives for the various organophosphorus pesticides and their transformation products.

The disappearance of the compounds and the estimated values calculated from GC-NPD and LC-DAD analyses, during a period of 5–6 weeks, are shown in Table 1.20. The loss of compounds is described by a linear regression of the logarithm of the area versus time. The initial values were based on the assumption that the initial concentration at zero time was 100%. k (first order rate constant) values are also re-

TABLE 1.20
HALF-LIVES ($t_{1/2}$) IN DAYS FOR ORGANOPHOSPHORUS PESTICIDES AND SELECTED
TRANSFORMATION PRODUCTS IN ESTUARINE WATER USING GC-NPD AND PROSPEKT-
LC-DAD: CORRELATION COEFFICIENTS (r^2) OF THE PLOT OF $\ln(\text{CONCENTRATION})$
VERSUS TIME

Compound	GC-NPD		Prospekt-LC-DAD	
	$t_{1/2}$	r^2	$t_{1/2}$	r^2
Chlorpyrifos-methyl	11.1	0.93	7.4	0.94
Diazinon	10.6	0.86	8.2	0.93
Disulfoton sulfoxide	10.4	0.97	12.3	0.99
Disulfoton sulfone	8.19	0.98	ND	ND
Fenamiphos	ND	ND	1.80	0.89
Fenthion	4.6	0.95	ND	ND
Fenthion sulfoxide	6.9	0.91	ND	ND
Isofenphos	11.9	0.89	9.8	0.90
Malathion	4.4	0.96	4.9	0.97
Methidathion	9.9	0.96	6.5	0.89
Ofunack	12.0	0.97	10.2	0.96
Pyridafenthion	11.5	0.98	10.8	0.98
Temephos	ND	ND	5.0	0.99

ND, not determined.

ported. From this table it can be seen that the values of $t_{1/2}$, determined using the two analytical techniques, differ by 6–30%, depending on the compound studied, but the rate constants did not differ significantly ($P > 0.05$). A concordance between the two series of results can be admitted since the precision reported for each technique is ca. 10%. The degradation kinetics of disulfoton sulfoxide, disulfoton sulfone and fenthion sulfoxide are also reported in Table 1.20. The values of $t_{1/2}$ determined for disulfoton sulfoxide and sulfone are 10.4 and 8.2 days, respectively, and 6.9 days for fenthion sulfoxide. These values are greater than reported for their parent compounds, especially for disulfoton which disappeared within the first week. Disulfoton sulfoxide and sulfone were quantified by GC-NPD at their maximum concentrations, with $37 \mu\text{g/l}$ (time 7 days) and $4 \mu\text{g/l}$ (time zero, just after spiking) respectively. Since the waters were spiked at a level of $50 \mu\text{g/l}$ of disulfoton we can assume that its degradation to its sulfoxide and sulfone is complete.

These results indicated that some compounds excluded from the National Pesticide Survey list (see Table 1.8), e.g., malathion, diazinon, and fenthion, have longer half-lives than some other organophosphorus pesticides included in the list, such as fenamiphos. Further studies are needed to establish a priority pesticide list for monitoring purposes that considers the various stability parameters depending on the environmental water type.

There is a problem in estimating the half-lives of a compound in the environment, because depending on the environmental conditions of each site, different values of half-life will be obtained. Thus, major variations were observed in the values in Table 1.16, which indicates the natural disappearance of fenitrothion under various conditions [121]. The degradation depends on the natural photolysis conditions, temperature, pH of the water, and the presence of microorganisms. It varies with the physicochemical properties of the pesticide itself and the climatic conditions of the site that can favour photolysis, hydrolysis or thermal degradation. An example of this is seen for degradation of fenitrothion, as shown in Table 1.16. Several experiments were performed in Canada, Spain and other parts of the world, also using different water types. Discrepancies in the half-life were noted and are attributed to the physico-chemical parameters and climatic conditions of each place [121]. In general, it is very important to correlate laboratory data and environmental data using a variety of experimental conditions. This is the best way to discover the behaviour of pesticides in the aquatic environment.

1.5. TOXICITY AND ECOTOXICITY

As pointed out previously, in the selection of priority lists, one parameter to be taken into consideration is the toxicity of the compound. Such toxicity evaluations depend on the compound and its concentration in water, and should take into account both its human toxicity and toxicity to aquatic organisms. For drinking water, the Commission of the European Communities has fixed a level of $0.1 \mu\text{g/l}$ for individual pesticides and $0.5 \mu\text{g/l}$ for total pesticides. This is a very strict measure, and analytical methods still need to be developed for a variety of pesticides to comply with this Directive. The Office of Water of the US EPA has established drinking water regulations and health advisory levels for individual pesticides. A selection of the different health advisory levels, also referred to by the EPA as Maximum Contaminant Level Goals, is indicated in Table 1.21. There is a relevant question, which has frequently been discussed in Europe. Why, in the European levels are there so many restrictions on the maximum contaminant level of pesticides which are not based on toxicological considerations? A few groups, including manufacturers of pesticides, have asked the European Union to change the limits, especially for some ubiquitous contaminants such as atrazine that are often detected at levels above 0.1 ppb in drinking water. As can be seen in Table 1.21, the maximum level in the USA is 3 ppb . The philosophy behind the European Union policy is the so-called "principle of precaution". In this way, by keeping very strict restrictions in the drinking water regulations, there is a guarantee that the pesticide levels will not increase in the future and will eventually be lowered. We should indicate that some permissiveness exists in the regulatory bodies about the 0.1 ppb level, otherwise many water supplies would need to be closed down. Atrazine is probably the compound most studied in drinking water, because it is easily detected and is a ubiqui-

TABLE 1.21
HEALTH ADVISORY LEVELS ($\mu\text{g/l}$) FOR SELECTED PESTICIDES IN DRINKING WATER
(FROM US EPA)

Compound	Health advisory level ($\mu\text{g/l}$)
Alachlor	2
Aldicarb	10
Aldicarb sulfoxide	10
Aldicarb sulfone	10
Atrazine	3
Bromacil	80
Carbofuran	40
Chlorothalonil	2
Cyanazine	9
2,4-D	70
Dalapon	200
Dinoseb	7
Diquat	20
Endothal	100
Endrin	2
Glyphosate	700
Methomyl	200
Methyl parathion	2
Metolachlor	10
Oxamyl	200
Picloram	500
Simazine	4
Trifluralin	2

tous contaminant. In a way it can be considered as like the DDT of the 1990 s. A systematic exposure assessment process has been developed for evaluating and describing the distribution, within large populations, of human exposure to atrazine through drinking water in Ohio, Illinois, and Iowa [155]. This assessment indicated that atrazine exposure through drinking water does not represent a significant human health threat, based on the current understanding of atrazine toxicity. Exposure to atrazine above the lifetime health advisory level of 3 ppb did not exceed 0.25% of the assessed population in any of the three US states. Most of the population had exposure concentrations lower than 1 ppb.

From the values in Table 1.21, we can see that other widely applied pesticides, such as glyphosate, have very high maximum contaminant levels in the USA, thus indicating that they pose very low risks for drinking purposes [156]. In such cases there is quite a big difference between the US and European levels, and some action will probably need to be undertaken in this respect by the European Union. There is not only a toxicological point of view but also an economic reason; it requires much more investment for official laboratories to monitor glyphosate at the 0.1 ppb level in

drinking water than at the 700 ppb level. The analysis of this compound requires a specific method which will require optimization for it to be performed at the 0.1 ppb level [15].

One of the major difficulties in estimating the toxicity of pesticides in the environment is in their ranking. There is no consensus on how we can weigh qualitatively different risks such as ecosystem health versus human health. For example, some pesticides will present higher risk to human health and others to aquatic life. In view of all the different factors that affect the ranking of pesticides, in California recently there has been established the so-called multi-attribute ranking model [157]. This model uses 13 criteria, among which are toxicity to beneficial arthropods, and acute toxicity to farm workers, bees, and birds. Toxicity to consumers, and ground water contamination, receive lower weights in this model. The question that this model addresses are whether one pesticide is less toxic than another and less toxic to whom. Table 1.22 shows the Hazard Ranking list of pesticides established in California, considering human health impact, natural resource impact, and the multi-attribute impact ranking. We can see that the ranking is different for all cases, although some common pesticides such as chlorpyrifos are present in all the rankings.

Ecological risk assessment is often used to establish whether a pesticide poses a problem to the water quality, and what measures should be undertaken to deal with it. Recently, an extensive review of atrazine risk assessment has been published in the USA [158]. The risk assessment formulated in this review was a three-step process. In the first instance, the problem formulation was pointed out, with stressor characteristics, the ecosystem potentially at risk, and the ecological effects with indications of the conceptual mode. In the second step was the analysis, with characterization of the exposure, the probabilistic assessment of residue-monitoring data for atrazine and its metabolites in the streams, rivers and reservoirs of major corn-

TABLE 1.22
HAZARD RANKING FOR PESTICIDES IN CALIFORNIA

Rank	Human health impact (farm workers)	Aquatic life	Multi-attribute impact
1	Sulfur	Trifluralin	Sulfur
2	Propargite	Chlorpyrifos	Copper hydroxide
3	Glyphosate	Propargite	Chlorpyrifos
4	Methomyl	Azinphos. methyl	Propargite
5	Chlorine	Endosulfan	Cryolite
6	Chlorpyrifos	Diazinon	Dimethoate
7	Parathion	Methyl bromide	Chlorothalonil
8	Methyl bromide	Permethrin	Maneb
9	Aluminium phosphide	Methomyl	Diazinon
10	Mevinphos	Carbofuran	Copper sulfate (basic)

producing areas, and also the characterization of ecological effects with the probabilistic assessment of laboratory toxicity, including studies with aquatic organisms. This included the characterization of multi-species laboratory and field studies and the results of microcosm and mesocosm studies. In a final step, the risk characterization was undertaken, with the comparison of species sensitivity and surface water exposure distributions. This final step included the consideration of uncertainty, confounding stressors, and ecological relevance. Based on all the data available, and a great number of experiments, it was concluded [158] that atrazine does not pose a significant risk to the aquatic environment. Although some inhibitory effects on algae, phytoplankton, or macrophyte production may occur in small streams which are vulnerable to agricultural runoff, these effects are likely to be transient and rapid recovery of the ecological system is expected. A small group of surface waters, principally small reservoirs in areas with extensive use of atrazine, may be at greater risk from this exposure. Therefore it is recommended that site-specific risk experiments be conducted at these sites to assess the possible ecological effects in the context of the uses to which these ecosystems are put and the effectiveness and cost-benefits of any risk mitigation measures that may be applied.

The Microtox System has been used for assessing the toxicity of pesticides. This system was originally developed to help assess the toxic effects of complex industrial effluents. However, since its introduction, the application of the Microtox System has been extended to the determination of the toxicity of aquatic pollutants, waste waters, and fossil-fuel process waters. Somasundaram et al. [159] used the Microtox System to assess the toxicity of pesticides and their hydrolysis metabolites. By using this toxicity test it was shown that many hydroxylated metabolites of pesticides are more toxic than the parent compounds, and this is true in many cases. It is therefore always very important to determine the pesticide transformation products formed in the environment, since they, too, may be more toxic than the parent compounds.

Endocrine-disrupting pesticides have been discussed in the literature in recent years [160]. Among the pesticides considered to exhibit reproductive and endocrine-disrupting effects are atrazine, carbaryl, methomyl, aldicarb and many organochlorinated pesticides. Information is still lacking on whether these chemicals may be responsible for endocrine-disrupting events. It is also argued that the cumulative effects of these chemicals might not be recognized until young adulthood, when dysfunction in the reproductive systems might become apparent. Another factor that may be considered in the future in evaluating the ecotoxicological impact of pesticides is DNA damage. So far, studies have been reported for marine pollution and a significant correlation was observed between the fraction of double-stressed DNA and the concentration of low-chlorinated biphenyl congeners substituted at the *meta*- and *para*- positions of the biphenyl skeleton [161]. No studies have been undertaken on the new generation of pesticides, to our knowledge, but such studies may open a new window in the ecotoxicological risk assessment of pesticide pollution.

1.6. CONCLUSIONS

In this chapter we have discussed general aspects of pesticides, from their physicochemical properties to their relevance in the environment. We have also indicated areas that are addressed later in greater depth. By knowing the problems of pesticide transformation in the environment, it should become easier to develop appropriate analytical methods and to find solutions.

It is known that most of the polar pesticides are transformed in the environment and that generally, the transformation products are more polar. Thus, although a pesticide may itself not be toxic by and may be less persistent, the transformation products can be much more toxic and more persistent. Furthermore, whether we can detect the pesticides in the environment depends on the analytical method that is being used. Several of the major pesticides used worldwide, such as glyphosate, are not being measured because they need a "tailor-made" analytical method, which is not included in most of the multi-residue approaches used in surface water monitoring. The data published in the literature therefore reflects the state of the art of the analytical techniques and the quality of training in the laboratories involved. Research, and routine monitoring are needed for specific or unusual pesticides and also in the determination of polar transformation products. Although new information has become available on pesticide transformation products in recent years, still more is needed. With the advent of new instrumentation, such as liquid chromatography, mass spectrometry with atmospheric-pressure chemical-ionization interfaces, and the use of new polymeric sorbent materials that can trap very polar analytes, the information on polar pesticide metabolites has increased. Information on very polar metabolites, such as the sulfonic compounds derived from alachlor and metolachlor throughout the waters of the USA, can change the ideas of monitoring that were current during the early 1990s [162]. Information that can now be obtained on the metabolites found in the environment can change the conclusions from past monitoring surveys. This may open up new areas for research and development [10].

Finally, concern about endocrine-disrupting chemicals has increased in the last few years. Several pesticides are among these, and further research will be required. The correlation of endocrine-disruption data with those from the monitoring for toxicity of pesticides and their transformation products, in the same experiments, could bring about integrated research studies, and solve some problems in the future in the area of pesticides and their metabolites in water. In the following chapters the various methods of analysis for the monitoring of pesticides and their metabolites in water are discussed.

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CHAPTER 2

Quality Assurance Issues: Sampling, Storage and Interlaboratory Studies

2.1. SAMPLING

2.1.1. Introduction

The selection of sampling procedures is at the basis of environmental studies and is often guided by information on the compound's persistence and their physico-chemical properties, e.g., K_{oc} and K_{ow} , vapour pressures, the regulatory needs, and the assessment of ecological impacts. Generally, there is a demand for data on the occurrence of a pesticide or metabolite in the "whole" water samples.

In this chapter we discuss matters related to the partition of the pesticides between dissolved and particulate matter, the importance of the filtration step prior to concentration, and the presence of colloids. Most problems that occur are related to adsorption on sampling tubes, bottles, filters, suspended material, and colloidal organic carbon. For bottles, materials such as glass, stainless steel, or Teflon are recommended and amber bottles help avoid photochemical degradation. Even when all the precautions are taken, the sampling bottles used for transport and storage of pesticides can cause losses through adsorption to the wall when $K_{oc} > 10^4$ – 10^5 .

All these factors need to be taken into account when the sampling of pesticides from water matrices needs to be programmed. Other considerations include the sampling strategy, to take account of temporal and spatial variability. However, although it is often believed to be relatively easy to carry out sampling it is, in fact, one of the most difficult operations in the whole analytical protocol. Let us imagine that we have planned to sample for chlorotriazines in surface waters; that we will do sampling only on the dissolved phase, and we will not care about particulate matter. After some time, for research needs or regulatory purposes, we want to know something about the more lipophilic compounds in a certain river. We will then need to carry out another sampling protocol, and although the extracts from the previous sampling have been stored well, they cannot be used for the second question we are

asking. As a consequence, particulate matter will need to be sampled again. In other words, once the sampling is done we can look at the compounds and the specific variability for which it was designed, but nothing else. The samples from a sampling are unique and the information will refer only to these particular samples. When another sampling protocol is performed, the information will be different.

In this introductory section, we draw attention to mistakes that commonly occur in university and research centres. The new student in a laboratory is asked to perform the sampling and pretreatment of the samples. That is, the most difficult operation (or the operation that can lead to irreversible errors) is undertaken by the least trained scientist. It is known that, in general, the senior Ph.D. student prefers to use highly sophisticated instruments incorporating computer devices, which seems to be a “more important” task in a research laboratory, and the sample treatment is left for the newcomers. It is a duty of the directors of laboratories to change this behaviour, otherwise, before the samples are injected into the gas or liquid chromatograph the data will be wrong. In this respect we assert that the sampling cannot be considered a separate analytical step, but is a key part of the whole analytical protocol. After sampling, the sample storage, sample pretreatment, and final analysis including final quantitation of the samples, will all validate our analytical protocol. It is clear that all these operations should be controlled by an accurate programme of quality assurance and that none of them can be treated inadequately, otherwise the final results will not be acceptable.

Recently, some guidelines on quality control in sampling for water analysis have been reported [1]. Some specific control tests considered were that there should be routine tests on the effectiveness of the cleaning of sampling vessels and sample containers. In this way, if contamination problems are revealed, they can be rectified before sampling. Secondly, field blanks should be used to provide routine checks on contamination, typically using deionized or distilled water, which should be subjected to the same preparatory steps as are applied to real samples. Third, field check samples should be taken to provide routine checks on sample stability. This is a key issue, and it is good practice to prepare check samples having known pesticide concentrations and to treat them, as far as possible, in exactly the same way as real samples. The use of duplicate samples to provide routine checks on sample stability, and routine charts of field blanks may be valuable ways of monitoring the control of sample contamination.

This chapter reflects the personal experience of the authors. Sampling has often been treated as a “black box”, and many protocols are used which originated several years ago. In many laboratories, the sampling protocols seem to follow an “oral tradition” related to the experience of the laboratory or to colleagues in that field. Here, we give some critical guidelines on the topic, which should be useful for researchers working on the environmental sampling of pesticides in water matrices. A general book on this topic was published some years ago [2].

2.1.2. Sampling plans

Perhaps the most critical element in environmental analysis is the sampling and analysis plan. The quality control criteria with sampling should be designated so that, where possible, the data produced will identify sampling errors and will show that these are effectively minimized and defined. Project personnel should tailor the quality control of the sampling programmes to the scope of the sample collection activity and should consider the physical state and location of the site, the sampling equipment, health and safety, and the maintenance of sample integrity. The quality-control programme should consider using bottle blanks, trip blanks, field duplicate samples, and equipment rinse samples. Adequate volumes of samples should be collected to ensure that the laboratory can analyze matrix spikes and the matrix duplicates that are indicated under the quality assurance management plan. One of the primary objectives of a sampling plan is to ensure the collection of representative samples. Representative sampling will help to develop environmental data that are both accurate and precise in relation to the sampling site. Sampling accuracy is usually achieved by incorporating some form of random selection into the sampling process, and an appropriate number of samples should be collected to generate a sufficiently precise estimate of the true mean concentration of the pesticide. Consideration should also be given to the use of statistical techniques to develop a confidence interval for sampling accuracy and precision.

The sampling program must ensure that the information meets the objectives of the measurements. There is little or no point in the outline implementation of a programme that is incapable of providing this information. The objectives are paramount in governing the resources and effort required for the sampling and analysis. The initial set of objectives should be designed carefully and the various steps are summarized in Table 2.1. In summary, the completed sampling plan must answer the following questions:

- What do we want to know?
- Why do we need this information?
- What happens to the results?
- What action may follow?

To give an example of a sampling plan, a checklist of the most relevant factors for consideration when performing sampling from a reservoir was reported recently [3] and has been adapted in this review.

- Read the sampling plan and any supplementary instructions.
- Find out whether legal permission to take samples has been obtained. Is the sampling really necessary?
- What special safety equipment and clothing are needed?
- Do we need a boat or life jackets?
- Can we do the sampling from a bridge? Generally, the middle of a bridge is ideal, and very practical, for avoiding interference from the river bank. The site

TABLE 2.1
SAMPLING PLAN OBJECTIVES

(i)	Definition of objectives. Aims and measurements
(ii)	Select target pesticides and analytical methods
(iii)	Determine sampling locations
(iv)	Fix number of increments and method of sampling
(v)	Select methods for sample preservation and pretreatment
(vi)	Prepare final plan. Review in the light of experience

must be checked carefully and, if a long-term monitoring study is performed (over 1 or 2 years) samples from the same points should be collected to permit comparative studies.

- Sampling in rivers, is generally made at mid-channel and a depth of between 0.5 and 1.0 m. On other occasions, the surface microlayer ($100 \pm 50 \mu\text{m}$) will be needed for sampling.
- If there is a pipeline, this should be flushed to ensure a constant composition prior to the sample collection.
- The effect of currents and the presence of underwater objects (rocks, mud, debris) must be considered at all times.
- Discrete or sequential sampling taken at constant increments of time or flow may be required. For example, the rapid degradation of the organophosphorus pesticide fenitrothion [4] and temephos [5] has been followed after manual or aircraft application, in estuarine waters, with sampling periods of 2–3 h. In this way, real half-lives can be calculated and knowledge gained about the concentrations of toxic organophosphorus pesticides and their metabolites. In other cases [6], sampling every month will be enough to provide the levels of carbamates and their metabolites in well water, during the whole year, at various locations of the wells.
- Are the samples to be combined? This was the case in the analysis of several water samples inside a rice-crop field [5]. Waters containing temephos after aircraft applications to the rice field were mixed together. At the time it was relevant to know the amount of pesticide that reached the crop-field waters during aircraft application, so the collection of composite samples was relevant. In other cases, such as the sampling in well waters [6] monthly, at different wells, combination of samples from different wells is inappropriate because the information on contamination from the cultivated land to specific wells would be lost. Other aspects such as preferential channelling, and the composition of the land section above the well, will also affect the contamination of the ground waters. In this way, information about the leaching of a certain pesticide to a certain well can be achieved.

For the analysis of pesticides and trace organics we may need to avoid plastic

materials because they may contain phthalates. Borosilicate glass will be needed for water samples containing pesticides (using amber bottles, in many cases, to avoid photochemical degradation of the pesticides). Cleaning of the bottles prior to sampling, will generally be performed using concentrated acid (e.g., 0.05% HNO_3) or non-ionic detergent, followed by pure water, with final storage in a dust- and gas-free environment. Prior to sampling, a previous cleaning in the same location, several times with the water to be sampled, will also be required in some cases, but not always. We give some examples later.

We also need to decide which preservative needs to be added to the samples? Table 2.2 gives a summary of the methods proposed and actually in practice [7].

Finally, we draw attention to a number of other points about sampling designs and conditions for their use (see Table 2.3).

In conclusion, the sampling plan should cover all the points mentioned above and take account of the constituents we would like to measure. In addition to the target pesticides that we are searching for in a certain water, conventional parameters such as pH, conductivity, temperature, nitrates, dissolved oxygen, alkalinity, and total organic carbon should also be measured. This will lead us to a better understanding of the effects of pesticide contamination in water.

TABLE 2.2
CONTAINER, PRESERVATION TECHNIQUES, AND HOLDING TIME FOR PESTICIDES IN WATER GIVEN BY DIFFERENT AGENCIES SUCH AS EPA AND ISO (STORAGE TEMPERATURE 4°C)

Pesticides	Container	Preservation	Maximum holding time
Organochlorine	Glass	1 ml of 10 mg ml ⁻¹ HgCl_2 or 1 g/l ascorbic acid or adding extracting solvent	7 days 40 days after extraction
Organophosphorus	Glass	1 ml of 10 mg/ml HgCl_2 or adding extracting solvent	14 days 28 days after extraction
Chlorinated herbicides	Glass	Refrigeration, sealed, HCl to pH < 2 Sodium thiosulfate	14 days 7 days 28 days after extraction
Polar pesticides (method 4 NPS)	Glass	1 ml of 10 mg/ml HgCl_2 1 ml of 10 mg/ml HgCl_2	7 days 28 days
ETU (ethylene thiourea)		1 ml of 10 mg/ml HgCl_2	14 days

General rules: Sampling of 1 l of water, do not pre-rinse bottles with sample before collection. Seal bottles and shake vigorously during 1 min. Refrigerate samples until extracted. Protect from light.

TABLE 2.3
SUMMARY OF SAMPLING DESIGNS AND CONDITIONS FOR THEIR USE

Type of design	Conditions
Haphazard sampling	Very homogenous samples over time and space are needed.
Judgment sampling	Specific environmental samples are selected for their unique value and interest
Probability sampling	
Simple random	The simplest random sampling design
Stratified random	Heterogeneous samples can be divided into parts which are internally homogeneous
Multistage	Needed when measurements are made on sub-samples or aliquots of the field samples
Cluster	Useful when population units cluster together (e.g., fish) in soil and ground water contamination rarely occurs
Systematic	The method of choice when estimating patterns or trends of contamination over space
Double	Useful when there is a strong linear relationship between the variable of interest and a less expensive or more easily measured variable
Search sampling	Useful when historical information, site knowledge or prior samples indicate where the object of the search may be found

2.1.3. Sampling from rivers, lakes, and well waters

The chemical composition of a flowing liquid such as a river may vary according to changes in a number of parameters such as temperature, flow rate, and distance from the source, none of which can be controlled during the sampling. Because of this it is always difficult to draw conclusions from a single sample. The full information may only be available after a large number of samples has been taken and analyzed.

Surface waters include a wide range of different types: surface run-off, ditches, creeks, rivers, lakes, estuaries, seas, industrial areas, effluents, and piped water. There is no single procedure or device that is adequate for sampling such a variety of situations. An overview of the different details has been published in a book [8].

As mentioned in the introductory section, it is important that the procedure to be used for collecting the sample is carefully designed during the sampling plan. Contamination of the sample from the environment around the outlet of the sampling line should be avoided, especially when the components of interest are at trace levels. A general recommendation is that samples from a stream should, whenever possible, be collected 30 cm below the surface and at a similar distance from the bottom.

For discrete spot sampling “dip samples” a weighted bottle should be immersed at a suitable location and retrieved. For rivers, this is normally mid-channel at a depth of 0.5–1.0 m. Depth samplers permit discrete samples to be taken at pre-determined depths. It is useful practice to rinse the sample container two or three times before

collecting the sample. However this practice cannot be employed when the sample needs to be collected with a preserving reagent or when it contains material such as suspended solids, oils, or grease that may be adsorbed on the walls of the container. There are rivers with high contents of suspended solids and consequently by doing this operation two or three times before the final sample is collected, the container can easily be enriched with suspended solids.

If systematic sampling takes place in a lake or reservoir the locations should be planned, generally by dividing the surface into squares and sampling in each one of these. When there is no time to follow the systematic sampling, then random sampling can be performed and will allow us to find where the "hot spots" of the lake or reservoir are located. Later, systematic sampling in a selected location should be undertaken for a better estimation of the trends and spatial variability.

During the summer, as the surface layers or lake become warmer the density of the water is reduced and vertical stratification tends to occur, which progressively reduces the concentration of dissolved oxygen in the lower layers. The bottom layer may eventually become anaerobic, releasing other substances such as ammonia, iron, manganese, nitrates, and phosphate from the bottom sediments. In addition, the growth of algae in the surface-layers or water may cause changes in the pH, alkalinity, hardness, and in the concentration of ammonia, nitrate, phosphate, carbon dioxide and oxygen. In this respect, it is known [5] that in rice-crop fields there is a pH fluctuation in the flooding water from day to night. The pH of the water samples can vary between 7.5 and 9.0 from the early morning until noon, and the rice water temperature can change from 26°C (early morning) to 33°C during the day. Paddy water's pH follows a diurnal pattern resulting from photosynthesis and the respiration of algae. These types of change should also be considered when carrying out sampling since they will certainly affect the monitoring and degradation of pesticides under natural conditions. The water type, together with the physicochemical properties of a certain pesticide, may explain the observed degradation of pesticides for example, the presence of nitrates, which is quite high in certain well waters from Spain, induces the photochemical degradation of fenamiphos [9]. To gain as much information as possible about the water type and how the water composition can affect the stability of pesticides, the concentration of nitrates, pH, and conductivity are usually measured during monitoring programmes, together with the pesticide levels [6].

The collection of water samples from wells follows a protocol that includes purging the water standing in the well prior to the sampling. Usually the water is purged until the pH and conductivity remain constant. Various protocols of ground water residue sampling are described in a book [10]. Description of multilevel samplers and their pipes, of diameter between 38 and 50 mm, are given. The distance to the water table should also be defined when ground water monitoring is performed. In general, when ground or well water monitoring is performed it is very important to know the history of the cultivated field, crops, and chemicals used on the land which

is above the wells. This information is basic for one to know which pesticides or metabolites can be detected in the various wells and, consequently, for the final analytical protocol to be developed. This protocol should be "tailor made" for solving the particular problems associated with wells or the ground water located below cultivated fields.

The surface microlayer can be of importance in certain cases, as in a study of the fate of temephos in coastal waters [5]. With sea water, the sea surface is enriched in organic surfactants, metal complexes, fatty molecules, and oil contaminants. Therefore, the chemical composition of this film can be very different from that of the bulk water below. Representative sampling of this layer is very difficult because the film is only $50\text{ }\mu\text{m}$ thick, and the usual agitation of the sea surface or coastal waters hampers the deployment of samplers and the contaminants are differently adsorbed and desorbed from the samplers. Also since different samplers behave differently their results are not comparable. It is accepted that layers between $100 \pm 50\text{ }\mu\text{m}$ will give information on the chemical composition of this layer. The fast dissipation following application of the organophosphorus pesticide temephos indicates that most of the active ingredient is lost during treatment, as a result of drift and volatilization during spraying. Several factors have been considered which depend on the physicochemical data. Several data are in the literature on the water solubility (WS) of temephos, with values 0.001, 0.03 or 0.27 mg/l whereas the vapour pressure (VP) is 8.6×10^{-10} mmHg. Although temephos has a much lower VP than other organophosphorus pesticides, the parameter to take into consideration in experiments involving air-water interfaces is Henry's law constant (HLC) which can be used to estimate the evaporation rates of chemicals from water if one assumes that all the chemical is dissolved in water. The ratio VP/WS gives an excellent estimate for the HLC and can be used to evaluate the evaporation rate from a water solution more accurately than does the VP. A second problem to be noted with temephos is the disagreement in the WS values (0.001, 0.03 and 0.27 mg/l) which gives additional difficulties in estimating the HLC. When making the appropriate calculations, and because temephos exhibits a very low and uncertain WS, the HLC expressed in $\text{Pa m}^3/\text{mol}$ of fenthion is 0.022, whereas for temephos it can be 0.54 if the solubility is 0.001 mg/l, 0.022 (if the solubility is 0.03 mg/l), or 0.002 if it is 0.27 mg/l. For comparison with other OP pesticides, dichlorvos, a very volatile OP, has a value of HLC of 0.19 and fenitrothion, which also suffers important losses in field application [4], has a value of 0.0036. As a consequence, the HLC for temephos indicates that this compound will have a tendency to volatilize from a water solution following aerial application. Also, as pointed out above, the extremely low water solubility of temephos may result in formation of a surface film of undissolved material. In this case, the HLC is not a predictor of the volatilization rate, and the vapour pressure is the more relevant parameter. As we can see, these two arguments seem contradictory, and reflect the complexity of sampling of pesticides for water bodies. With this example we can see that the surface microlayer can be of importance for the more lipophilic pesticides. In

general, we should look first to the physicochemical properties of the pesticides that should be monitored in the environment. After looking through the data, the sampling protocol and the frequency of sampling should be planned. This is fine for the so-called “target sampling”, where we know in advance which pesticides are being applied in a particular area, e.g., when there is pesticide spraying by plane. However, problems arise quite frequently when monitoring of river waters must be carried out, where we do not know which pesticides we are looking for. Certainly in this case we start with a certain type of sampling and then, after a few analyses, we may need to change the sampling strategy as a result of the qualitative data obtained. Sampling should not follow a rigid scheme and should be adapted and changed as a function of the different variables (compounds found, river flow, irrigation practices, etc.).

2.1.4. Sampling sea water

Apart from the microlayer mentioned earlier, the main point to be considered is that organic pollutants – and with pesticides, mainly the organochlorinated pesticides – will be present at very low levels in sea water. This means that methods should be developed for analyzing large water samples from sea water for pesticides.

For deep-sea water – which will mainly be of interest for organochlorinated pesticides – Nyskin-type bottles are used. For sea water or coastal surface waters, where the concentration of organochlorinated pesticides needs to be measured at very low levels, the collection of samples in large reservoirs of 100–500 l followed by liquid–liquid extraction (LLE) procedures, is common practice [11,12]. In the LLE procedure, the solvent employed is generally hexane, pentane or chloroform, solvents which are not significantly miscible with water. The Goulden large-scale sample extractor was used for this purpose. Up to 120 l of water can be processed and it is ideally suited for the analysis of pesticides in streams because of the requirements for depth and cross-sectionally integrated sampling regimes. The entire sample must be collected, filtered, and composited before extraction, making rapid extraction techniques desirable in water-quality surveys. The Goulden large-sample extractor functions as a single-stage mixer/settler, in which a continuously flowing sample is extracted by a fixed volume of stationary methylene chloride. By using this device, not only organochlorinated pesticides gave good recovery values, but also metribuzin, atrazine, linuron, methyl parathion and permethrin. Field-extractions using a flow rate of 340 ml/min were performed. At higher water volumes (120 l) the recoveries were lower, but at 40 l the recoveries were still acceptable for most of pesticides reported.

Some of the topics above have been discussed in a review article [13]. The various methods of extraction, including bath solvent- extraction, continuous LLE, pressurized extraction and filtration, and solid-phase extraction were briefly discussed. The Goulden extractor is part of the continuous LLE devices. The systems developed by Hermans et al. [14] also use continuous LLE. In this technique the water is also

more exhaustively extracted. Batch solvent extraction is limited, primarily because of the difficulties associated with handling large volumes of water, ca. 20–40 l. Small volumes up to 4 l are easy to handle but higher levels become quite tedious. A pressurized system such as that described by Kelly et al. [11] represents an enclosed system which overcomes many of the problems in sampling in coastal zone areas. It does not distinguish between dissolved and colloid-bound contaminants. Samples up to 30 l can be processed, and particulates are collected in a stainless steel system, filtered through a $0.7\ \mu\text{m}$ glass fibre filter, and force-extracted with pentane using a high speed stirrer (300 rev./min). The last systems we consider are based on SPE. Estuarine samples of high salinity, with water volumes up to 4 l, were preconcentrated using Empore extraction disks and then used for the determination of chlorotriazine pesticides [15,16]. This method is very easy to use and permitted the determination of atrazine, simazine and de-ethylatrazine in a salinity profile down to levels of 5–10 ng/l. However, the methodology more frequently used for sea water samples, based on the SPE technique, uses the sorbents XAD-2 and XAD-4. The major disadvantage of this technique is the extensive extraction and manipulation of the sorbent needed to obtain a clean resin. This aspect, although not mentioned for LLE, is very significant. Good blanks should always be obtained and are very important for sea water samples, since the levels monitored are very low. Reagent blanks are of extreme importance and the quality of the extracting solvent should always be checked. The SPE columns can be sealed and mounted in a glass water-sampler such as the Seastar Instrument, (Sidney, Canada). Such a sampler has been used to extract up to 435 l in open ocean water. The analytical protocol generally followed includes filtration at 0.7 and then $0.45\ \mu\text{m}$, addition of internal standards, preconcentration of the analytes from 20 to 40 l of sea water samples at a flow rate of 100 ml/min, washing of the system with twice distilled water, drying of the sorbent bed, and elution of the analytes using an appropriate solvent. This commercial system is being applied in several monitoring programmes and, since it uses commercial equipment, modifications on the chemical composition of sorbent can always be carried out. A modification that uses 90 mm Empore disks was used for preconcentration of a number of organochlorinated pesticides and chlorotriazines. The use of such SPE devices seems to be the best solution for the handling of sea water samples. One of the key problems remaining in such experiments is the variability of breakthrough volumes: when large volumes of sea water samples are used at high flows (ca. 100 ml/min) the breakthrough volumes of certain analytes may be reduced. All the parameters necessary should be carefully optimized in laboratory experiments before going to the field, at least using artificial sea water samples spiked with the target pesticides to be analyzed.

In summary, the sampling of polar pesticides from sea water samples is a relatively small problem, mainly because few classes of polar pesticides are known to reach coastal sea waters. One class that does is the chlorotriazine pesticides which are currently measured in various estuarine and coastal waters following their trans-

port through the dissolved phase of the rivers and homogeneity mixing in the estuaries. Other classes, such as the organophosphorus pesticides, are known to degrade easily and do not reach sea water. For this reason, most of the research and application of sampling methods to sea water samples has been restricted to organochlorinated pesticides.

2.1.5. In situ preconcentration

Automated methods, such as the system for the automated monitoring of organic pollutants in surface water (SAMOS) used in the Rhine basin programme for pesticide monitoring which uses automated on-line SPE-LC-DAD or GC, have been applied extensively. Several papers dealing with this procedure have been published [17,18]. These stations automatically collect water samples from the river, with a filtration device, and on-line preconcentration with further analysis by either by GC or LC techniques. In some instances, when the river particulate is high, prefiltration devices of 10 and 1 μm are used before the sample is analyzed in the SAMOS. We do not discuss the use of such automated on-line SPE systems since it is not the objective of this chapter.

We do want to indicate that there is research into the development of “alarm” systems for detecting pollution of rivers by pesticides. For river water that will be used for drinking purposes, the SAMOS system can detect pesticides in real-time and allows one to follow an accident or spillage into some part of the river before it enters the treatment plant. The chromatographic profile of the water will change gradually from one monitoring station to another, and will reach the background level after a certain time, indicating a contamination profile through the river. The implementation of such in situ sampling methods is quite expensive in both the equipment and the trained personnel that are needed.

Other techniques that can be applied to monitor, in situ, the pollution by pesticides in river water use immunoassays and biosensors: these have been reviewed recently [19,20]. There are commercially available portable immunoassay devices that can work for up to 9–10 h with a battery and permit the detection of the target chemical, using antibodies. The use of immunoassays in situ combines simultaneous sampling and analysis; no trace-enrichment procedure is needed since most immunoassays now need only 0.2 ml of water sample and give a sensitivity of 0.1 ppb. The immunoassays are discussed further in Chapter 6. The major weakness of immunoassays for screening and “alarm” purposes is that there do not exist multianalyte immunoassays that permit the simultaneous screening for a variety of analytes in a river. There are immunoassays which are specific for individual chemicals, or, in some cases, for a class or group, but not for different groups of pesticides. Certainly, so far there are not enough immunoassays for such purposes. A recent review deals with those that are commercially available [21]. Immunoassays have been developed and applied widely for certain pesticides, such as atrazine and related compounds. Many moni-

toring programmes in Europe [22] and the USA [23] are using immunoassays for the routine monitoring of atrazine and give excellent correlation with chromatographic techniques. Certainly, one way of solving the sampling-sample pretreatment problem, is to “use direct sampling and no sample pretreatment”. In this respect, immunoassays offer the possibility of carrying out relatively easy samplings from which the analytical results of up to 90 samples, can be obtained 1 h later. This is what the regulatory agencies and many researchers would like to have. Unfortunately, not all the in situ devices are so easy to implement, and not all chemicals can be measured with the same degree of accuracy. The application of biosensors follows a similar approach but their use in environmental pesticide analysis is limited and further research is needed. The development of immunosensors is very much dependent on that of immunoassays and consequently such developments will go hand in hand. The area of in situ measurements in the field will need to grow since many problems related to field environmental sampling need to be solved. The agreement between immunoassay techniques and conventional gas chromatography–mass spectrometric methods can be seen in Fig. 2.1, where the levels of total triazines and atrazine were measured by both methods independently, with a high degree of correlation [22].

Other methods for measuring in situ contaminants in water samples use the so-called lipid-containing semipermeable membrane devices [24,25]. These allow passive in situ monitoring, and have been applied mainly for persistent organic chemicals, especially PCBs. The device usually consists of a thin film of neutral lipid (molecular mass > 600 kDa) such as triolein, enclosed in a thin-walled lay-flat tube made of low-density polyethylene or other non-porous polymer. Model estimation and laboratory experiments show that it is feasible to use this device to determine average concentrations of organic pollutants in natural waters. Other methods of in-situ direct analysis include the use of membrane-introduction mass spectrometric devices which permit the direct analysis of water samples from rivers. These devices have been applied mainly for volatile compounds but could also be applied for pesticides in water [26]. In conclusion, this area of in situ sampling is developing and we believe that it will grow in the next few years, mainly because there is a need for such field instrumentation devices, based on biological techniques with multi-analyte immunoassays, biosensors, or chemical devices. When sampling is performed, the sample is changed, to a minor or major degree, and procedures for direct sampling analysis in the field will be developed to avoid such alterations and to achieve real-time monitoring data.

2.1.6. Organic matter

The organic matter in natural waters covers a continuous spectrum of size, ranging from free monomers, via macromolecules and colloids, to aggregates and large particles. Terms frequently used are the colloid organic carbon (COC), dissolved organic carbon (DOC), or total organic carbon (TOC). All these terms

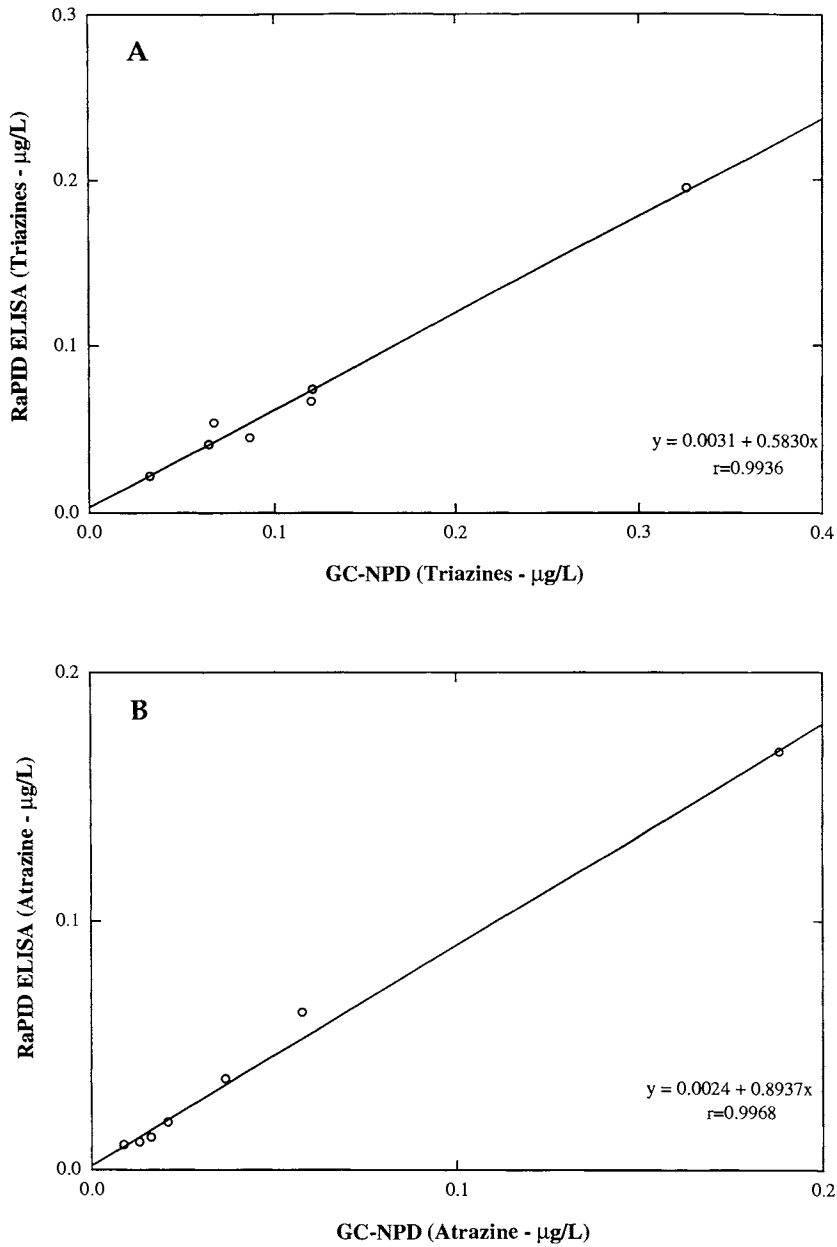


Fig. 2.1. Comparison of total triazines (atrazine and simazine) and atrazine concentrations in estuarine water samples from the Ebro delta (Tarragona, Spain) determined by GC-NPD and RAPID ELISA assay. The regression equations for the two lines are indicated.

may lead to confusion; definitions of the various terms have been given in a book chapter [27].

The DOC is generally defined as the organic carbon passing through a $0.45\ \mu\text{m}$ silver or glass-fibre filter and it is the most important term used in the study of organic carbon. DOC quantifies the chemically reactive fraction and gives the mass of organic carbon dissolved in the water sample. The suspended organic carbon (SOC) is the organic carbon retained on a $0.45\ \mu\text{m}$ silver filter. There are some advantages in using silver filtration because: (a) the size cut-off is between 0.1 and $0.45\ \mu\text{m}$, whereas the glass fibre filter gives a slightly greater size cut-off of 0.5 – $2.0\ \mu\text{m}$, so the larger particles of the glass-fibre filter may consist of suspended organic carbon associated with clay, and (b) the silver from the filter dissolves into the sample at a concentration of 50 – $100\ \mu\text{g/l}$ and acts as preservative to prevent bacterial growth. The disadvantage of silver filtration is that it is more expensive and slower than filtration through glass fibre filters. The particulate organic carbon (POC) is the organic carbon retained on a $0.45\ \mu\text{m}$ glass-fibre filter and is essentially identical to suspended organic carbon. The total organic carbon is the sum of DOC and SOC or the sum of DOC and POC. In general, it is important to separate the various forms of organic carbon. This is because: (a) DOC is chemically more reactive because it is a measure of individual organic compounds in the dissolved state, whereas SOC corresponds to organic coatings on silt and clay; (b) SOC and POC increase dramatically with increasing discharge, while DOC varies less; and (c) in many lakes, small streams and the open ocean, the concentration of suspended organic carbon is small ($<10\%$), and in these cases TOC is almost identical to DOC.

The DOC is usually measured by the persulfate/UV method and common values reported vary between 2 and $5\ \text{mg/l}$, e.g., in the Rhine and Niagara rivers. Common values of $5\ \text{mg/l}$ are generally reported for many rivers. Concentrations of DOC in rivers range from less than $1\ \text{mg/l}$ in alpine streams to more than $20\ \text{mg/l}$ in some tropical or polluted rivers and rivers draining swamps and wetlands. The following estimates, reported by Thurman [28], are given in Table 2.4.

By generally using the persulfate method, only the low-molecular-weight fraction

TABLE 2.4
DOC (mg/l) ESTIMATES IN VARIOUS RIVERS, ACCORDING TO THURMAN [28]

Location/type	DOC values and ranges
Arctic and alpine environments	2 (1–5)
Taiga	10 (8–25)
Cool temperature	3 (2–8)
Warm temperature	7 (3–15)
Arid	3 (2–10)
Wet tropical	6 (2–15)
Rivers draining swamps and wetlands	25 (5–60)

is determined, perhaps with some of the easily oxidized colloids. The low-molecular-weight material might show some consolvency, but it is not capable of adsorbing contaminants.

Catalytic oxidation at high temperature in the so-called TOC analyzer determines both organic fractions and shows results higher or equal to those above. It is probably the method most used nowadays. Because TOC also includes the low-molecular-weight fraction, these values cannot represent the COC. In this respect, it has been postulated that the difference between TOC and DOC may represent the COC [29]. A method for determining COC uses ultrafiltration and can be determined as the difference in carbon content of the total sample and the filtrate. According to Thurman [28], five geochemical processes are known to transform DOM in water: sorption/partitioning, precipitation, volatilization, oxidation/reduction, and complexation. The importance of the DOM and the interactions with different pesticides have been reported in a number of articles. One of the key issues in DOM is the composition of the DOC which is accepted to be formed by humic substances, hydrophilic acids, carboxylic acids, amino acids, carbohydrates, and hydrocarbons. The most important group discussed in the literature corresponds to humic substances, and especially their interactions with pesticides. Soil humic substances are the coloured polyelectrolytic acids that are operationally defined by their isolation from soil with 0.1 N NaOH whereas aquatic humic acids are defined as coloured, polyelectrolytic acids isolated from water by sorption onto XAD resins, or weak-base ion-exchange resins or some comparable procedure. They are non-volatile and range in molecular weight from 500 to 5000. Generally, aquatic humic substances have an elemental composition that is 50% carbon, 4–5% hydrogen, 35–40% oxygen, and 1% nitrogen. The major functional groups include carboxylic acids, phenolic and alcoholic hydroxyl groups and keto functional groups. The structure of aquatic humic substances is unknown. Humic substances generally divide into humic and fulvic acids. The humic substances that precipitate in acid are humic acids and those in solution are fulvic acids. In practice, this separation removes the larger aggregates of humic substances that originate from degradation of plant matter. Fulvic acids are more water-soluble because they contain more carboxylic and hydroxylic functional groups and have lower molecular weights, from 800 to 2000. Humic acid has molecular weight above 2000 and is often of colloidal size. Humic substances play an important role in the pollution of water. Historically, they played an important role as precursors to the formation of chlorination by-products such as trihalomethanes, and exhibit an important role in the concentration and transport of pollutants in rivers.

Some terms about the characteristics of water samples and the distribution of a chemical in the water and the various compartments have been reported [30].

(i) Dissolved in aqueous solution or freely dissolved: in this case, the pesticide is totally dissolved in the water fraction, which may be an ideal case in natural waters.

(ii) Associated with colloids: this association is usually through specific interac-

tions with clay minerals or the interaction between organic compounds and organic polyelectrolytes, such as naturally occurring humic, fulvic and acidic polysaccharides. The strength of the interaction is thought to be closely related to the molecular size and composition of the colloids and the intrinsic water-solubility of the pesticide. Generally colloids are defined as $<0.2\ \mu\text{m}$ in size and include many clay components which pass through $0.45\ \mu\text{m}$ membrane filters. For clay colloids, surface run-off, re-suspension of bed sediments, or river-bank-erosion are likely to contribute to the colloid component and so techniques are needed to assess each situation in the field. One aspect that needs to be considered, and is discussed in the following chapters, is that the efficiency of the extraction process, e.g., using SPE columns, may be altered by the presence of colloids as a result of the increased mobility of the colloid fraction through the extraction, column leading to “breakthrough” of the pesticide.

(iii) Suspended particulate matter (SPM): this is generally defined as all the particles that are retained by a $0.45\ \mu\text{m}$ filter [14], although other authors use the term to refer to particles of $>0.2\ \mu\text{m}$. Many organic compounds, particularly the CBs, and organochlorinated and pyrethroid pesticides, are strongly sorbed to sediment and organic particles. The larger size of these particles results in greater difficulties in obtaining representative samples, particularly when the suspended sediments are a major part of the suspended solids. For compounds with a moderate solubility and low octanol–water partition coefficient, such as the chlorotriazines, the amount of compound associated with the suspended solids is estimated to be less than 10%, so the compounds will be transported through the river in the dissolved phase [21]. When the transfer of chlorotriazines was investigated in a salt-marsh environment, it was found that atrazine and simazine entering the marsh system undergo negligible adsorption onto suspended solids. Herbicide accumulation within the sediment compartment was thought to occur by mechanisms, such as partitioning into the surface microlayer or association with sediment flocs. The majority of the herbicides were retained in the uppermost layers of the sediment profile, indicating that salt-marsh sediments act as a sink for these compounds. Atrazine and simazine entering the salt-marsh environment are predominantly in the solution phase [31]. In contrast, compounds like DDE and pyrethroids are estimated to be transported more than 70% into the suspended solids.

In consideration of the interactions between humic substances and pesticides, one of the most relevant aspects is the chemical structure of the pesticide. With comparing atrazine and chlorpyrifos, their nitrogen atoms will act as electron pair donors whereas the humic substances will act as (acceptor) electron-deficient quinone moieties [32]. In general, weakly basic compounds containing nitrogen groups should behave in a similar way to triazines in their interactions with humic substances. Previous experience with alachlor [33] which has a similar chemical structure to metolachlor, showed interactions with humic substances, and when the pH of the water changed from basic to acidic, alachlor was released from binding with the

humic substances after 15 h of contact. This was estimated to be around 5% of the total alachlor. Previous results [34] had indicated that alachlor does not bind to water-soluble soil organic matter (WSSOM), although the experiments were not reported. This is somewhat strange, since compounds with a similar K_{oc} value to alachlor (115–120 ml/g), such as simazine and atrazine, were found to bind to WSSOM. The binding of alachlor to humic substances was demonstrated by irradiating alachlor solutions during 15 h in order to ensure total photodegradation of the dissolved alachlor. However, changing the pH from 7 to 1 led to release of the pesticide. The alachlor fraction was bound to humic substances and was never available to photolytic action. The binding fraction was estimated at around 5% of the initial alachlor concentration. Binding by physical sorption is reversible, and Van der Waals forces are involved in these interactions rather than covalent binding from stable aggregates. The masking behaviour of humic matter was optimal within a contact time of 24 h before irradiation. In other cases, more days are needed and up to 5 days are needed for compounds of medium lipophilicity [32]. Pesticides that were found to bind to WSSOM were: paraquat, diquat, 2,4,5-T, simazine, bromacil, diuron, and glyphosate among the most polar pesticides [34]. An interaction of metolachlor and carbendazim with humic material was also observed with water containing humic substances when these pesticides were preconcentrated by solid-phase extraction material, using either cartridges or disks [35,36]. Similar experiments were performed with parathion-ethyl; with water containing 10 ppm of humic substances, approximately a third of the pesticide was not retained on the cartridge but formed a complex with the humic substance [37]. Parathion, which contains a nitro group and phenolic oxygenation shows interaction with humic substances. It was reported that the presence of the nitro group in the molecule is not sufficient for binding to organic matter and that this can occur only when both nitro and the oxy group are in the molecule. The charge-transfer interaction between certain substances and humic materials seems to be the most important feature and is also responsible for the physical bonding between pesticides and humic substances.

There has been interesting discussion about the contact time needed for a chemical to bind to natural humic substances present in rivers. It is accepted that for equilibration for spiking experiments, especially when using natural waters, it should be around 24 h. The time of equilibration is of importance if the association kinetics between the pesticides and the DOM is of the order of days, and not minutes or hours. In this respect, it was reported for the case of the pesticide mirex with humic acid water at organic carbon concentrations of 5–6 mg/l that, immediately after spiking, the mirex was totally recovered, whereas the recovery declined after 5 days. These data suggested that at least 5 days are needed for equilibration. It is generally accepted that the recovery of many organic chemicals in natural waters decreases as the time after spiking increases from 4 to 60 days [38].

Intermittent rain can influence the sediment load [36] of surface run-off from ag-

gricultural fields, thereby causing variability in the amounts of sediment and DOM in the water, which can adversely affect the extraction efficiency, and ultimately the method sensitivity in pesticide analysis in water. Studies using commercial humic substances, with humic acid concentrations varying from 0 to 25 mg/l, were performed. The effects of Ca-montmorillonite and humic acids were pH-dependent and acted independently in affecting the extraction efficiency. Lower recovery of most pesticides was noticed at pH 8 when Ca-montmorillonite was above >0.1 g, which was attributed to a greater dispersion of the clay, increased surface area, and subsequent adsorption. It was reported that the concentration of DOC in humic acid has less effect on the extraction efficiency when water was at pH 8 than at pH 6, which was probably caused by greater non-polar interactions between the pesticides and the charge-neutralized humic acid polymer. Of the pesticides studied, only the dinitro-anilines, pendimethalin and trifluralin, which have lower water solubility than the other herbicides (triazine, acetanilides and organophosphates) have a greater interaction with the non-polar surfaces of clay than with water. In general, the addition of Ca-montmorillonite reduced the pesticide recovery slightly more than humic acids, and this was attributed to the removal of pesticides associated with the clay through the prefiltering step.

In summary, the examples cited above indicate that natural waters contain organic matter in form of dissolved and/or particulate matter. The interaction between the organic matter and the pesticides depends on the chemical structure of the pesticide itself. In principle, if the pesticide is more basic, with nitrogen groups having free electrons that can interact with the quinone-like structures of the humic substances, then the effect of the humic substances with the pesticides will be higher. Another aspect to be considered is the contact time which will influence the physical binding of pesticides to the humic substances. The availability of pesticides which are bound to humic substances, and the operations needed afterwards to analyze pesticides, such as solid-phase extraction, are affected by the presence of humic substances. Research is currently being performed to study the behaviour of pesticides in the presence of humic substances.

2.1.7. Filtration

Filtration of water samples is a key operation but in many river waters is subject to adsorption on the filter material and clogging of the filter by suspended particulate matter (SPM).

An important factor to be taken into consideration is the type of membrane filter used; there are many types and pore sizes. A comparative study has been made between five different types: 0.2 μm cellulose nitrate (CN1), 0.2 μm cellulose nitrate boiled for 5 min in 400 ml of distilled water and freeze-dried overnight in order to reduce the amount of wetting agent on the filter (CN2), 0.2 μm Anopore inorganic membrane, 0.7 μm GF/F glass fibre pretreated to remove organic carbon by heating

to 500°C overnight, and 0.4 μm Nuclepore filters. These were compared for the filtration of organochlorinated, pyrethroid and triazine pesticides at concentrations of 0.25 ppb [39]. It was observed that, for the organochlorinated pesticides, the adsorption behaviour correlated well with the octanol–water partition coefficient. The triazines were not adsorbed on to glass or PTFE whereas α -BHC, lindane, dieldrin and endrin were adsorbed weakly as compared to DDT, DDE, TDE, permethrin, cypermethrin, and fenvalerate. Figure 2.2 illustrates these examples and shows the behaviour of the five different filters tested for the various pesticides, with the percentage of removal by each of the different filters (per = permethrin, fen = fenvalerate).

In the same work it was shown that adsorption by containers was a very important factor to be considered when analyzing compounds such as pyrethroids and organochlorinated pesticides. In accordance with their high octanol–water partition coefficients ($\log K_{ow} > 5$), approximately 10% or more adsorption took place on to the container wall of a 1 l bottle. In general, it is accepted that a truly dissolved compound exhibits a $K_{ow} < 10^4$ and quantitative adsorption of pesticides can already take place for pesticides exhibiting a K_{ow} value of 10^3 .

In summary, separation of the suspended solids by filtration is possible by careful choice of membrane filters and making a detailed mass balance, taking into account the retention of pesticides on the filter matrix.

Hermans and co-workers [14] also showed the importance of using various filtration systems for the analysis of CBs. Apparently they showed that the lower chlorinated CBs were represented more strongly in GF/F than the higher chlorinated CBs, expected on the basis of their aqueous solubilities (the lower PCBs exhibit higher water solubilities than the higher CBs, so it is expected that their concentration in water will be higher). Since the concentration difference in solubilities is often around 100 times, and the concentration found in the filtrate was two times higher, it was concluded that the higher chlorinated CBs were generally associated with dissolved organic matter (DOM). With membrane filtration, a pattern intermediate between that from GF filtration and centrifugation occurred, but with a much lower CB content. In this case, the differences were attributed to adsorption of freshly dissolved CBs on to the membrane filters. Consequently, membrane filters appeared to adsorb all freely dissolved CBs while GF/F showed a limited adsorption.

The distribution of various pesticides between water and suspended sediments in San Francisco Bay, CA, has been reported in an interesting article [40]. The relatively hydrophilic pesticides chlorpyrifos and diazinon had a uniform concentration in suspended sediments. These pesticides were detected after spring rains. Most of the measured diazinon was in the dissolved phase (at least 98%). Measured partition coefficients for diazinon were generally uniform, which suggests that the suspended sediment concentrations were closely in equilibrium with the dissolved concentrations. Partition coefficients could not be measured for chlorpyrifos, because the dissolved chlorpyrifos was found in levels below the detection limit. Carbofuran, the other pesticide detected, was only found in the dissolved phase. Certainly, these data

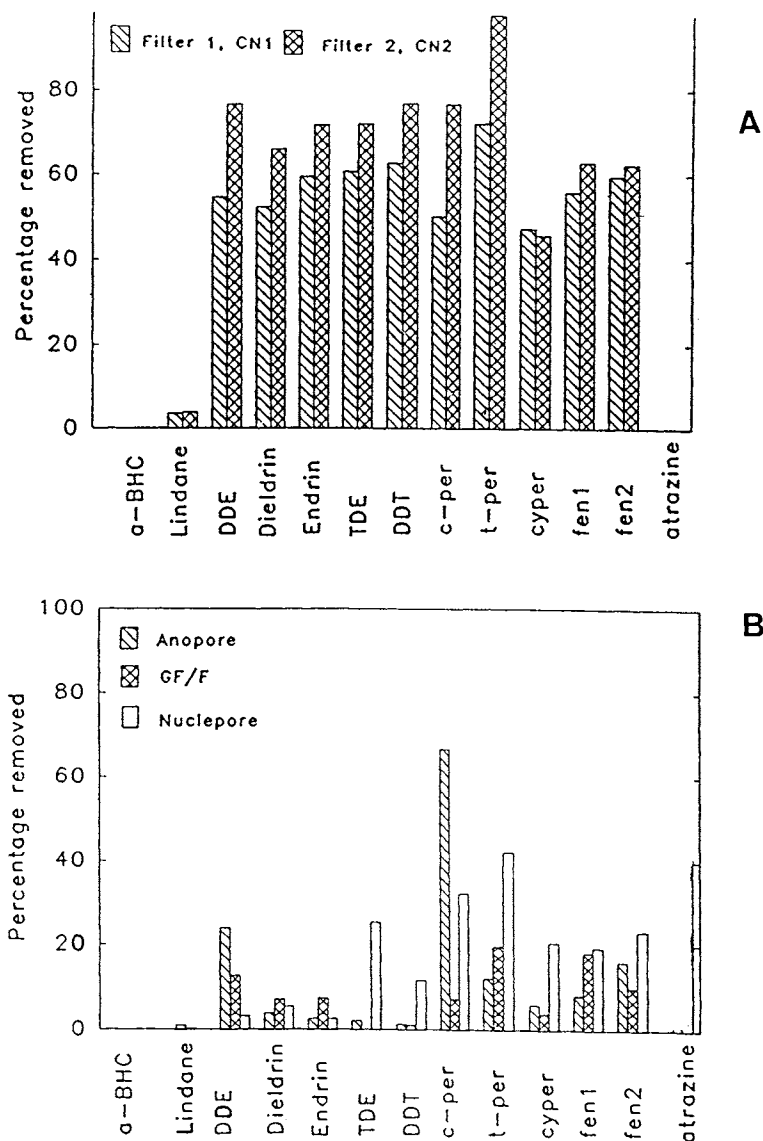


Fig. 2.2. Removal of pesticides after passage through membrane filters, expressed as percentage of the total addition minus the amount measured on the glass. The results indicate that cellulose nitrate membranes (a) do not retain α -BHC, lindane or atrazine, whereas the inorganic filters, GF/F and Anopore (b) retain smaller amount of a wide range of pesticides. Reproduced from Ref. [39].

correlate well with the K_{oc} values of carbofuran, chlorpyrifos and diazinon, of 2.07, 3.47 and 2.76, respectively.

The experimental data discussed above lead to general conclusions about the use

of filtration systems in the analysis of pesticides in water, make a good selection of the filtration system and use always the same type, manufacturer, and pore size during the whole monitoring period. Certainly, if a survey programme is performed, samples analyzed during the whole year need to be compared. The consistent use of the same filtration system is needed for comparable results to be obtained. By changing the filtration system, it can be clearly seen in Fig. 2.2 that the variability observed in the analysis of samples could be attributed to the filtration and not to the sample itself.

In summary, before planning the sampling of pesticides in water, the filtration must be designed according to the target analytes to be determined. If, by any chance, the analytes determined or found are different from those previously targeted, the filtration will also need to be changed. This is a critical point, and a careful study of recoveries will need to be undertaken if the filtration system is changed during the monitoring period. In general, it is better to always keep the same type of filters and manufacturer throughout the sampling period, without change.

2.1.8. Conclusions

There is no fixed recipe for sampling. Make your own sampling plan before sampling. Look at the type of water matrix to be sampled and the compounds you expect to determine. Check the physicochemical parameters of the pesticides and draw conclusions about the equipment needed. Also, make some laboratory experiments. For example, simulate a degradation experiment in a closed vessel and compare the data with those from field work; generally, under field conditions, degradation is faster, but the formation of metabolites should be similar. Use the same water type, conditions, sunlight, and concentrations of exposed pesticide since the degradation is dependent on the analyte concentration, among other parameters. Analyze the water for other parameters, conductivity, pH, and organic matter, since many of these can be of importance in the distribution and fate of pesticides in water.

The indications given should be of help for planning and performing the sampling programme and, what is more important, once a method has been discussed and approved, it should be followed during the whole monitoring period. Method improvements should be performed, but one should always keep in mind the fact that the results obtained should be comparable to previous ones, either directly or by applying a correcting factor.

Finally, use an appropriate reference material, once the sampling protocol has been finalized. This is quite difficult for pesticides, since no reference materials are yet available. However, it is possible to participate in interlaboratory studies using distributed calibrated samples. In this way we can investigate some parameters of the sampling protocol, for example the filtration.

2.2. STORAGE

2.2.1. Introduction

In the past, much effort has been wasted on the analysis of a variety of samples of doubtful integrity and it has frequently been observed that valid analysis depends upon reliable sampling and storage. Methods for collection and preservation are particularly important in the analysis of traces of pesticides in environmental samples. Maskareinec et al. showed that acidification with HCl effectively prevented degradation of volatile compounds and allowed sample storage for 112 days [41]. The National Pesticide Survey (NPS) and the US EPA state that all monitored pesticides included in their programmes should be stable in water for at least 14 days, after being inhibited biologically at $\text{pH} < 3$ and stored at 4°C [42–46]. Organophosphorus (OP) pesticides have exhibited many stability problems and many of them have been eliminated from the NPS list (see Table 2.5) because of this. The US EPA has withdrawn parathion-ethyl and -methyl, azinphos-methyl, fenitrothion, demeton, fenthion, and malathion from their list because of instability [47], although they are still included in EU lists. In a recent paper [48], it is reported that few organophosphorus compounds, some of them common to the US EPA list, were stable at 4°C in water from the river Axios in Greece at $\text{pH} 8$ over 8 days (see also Table 2.5).

In some instances, the degradation products of pesticides are more stable than the parent compounds so analysis should be aimed at the breakdown products [49–51]. In general, the half-lives of pesticides at low $\mu\text{g/l}$ concentrations are, as one might expect, very much dependent on storage conditions (pH , exposure to light, and temperature). Biological degradation and adsorption on particulate matter are also important factors [49,52]. For some compounds, degradation can be rapid. Studies on the degradation of carbamate pesticides in water have shown that loss can take about 20 days for methiocarb sulfone, methiocarb sulfoxide and 3-ketocarbocofuran [53], whereas, for carbaryl, losses can approach 90% in 1 day [50,51]. In general, samples should be analyzed as soon as possible after collection.

In summary, losses of pesticides in water can be due mainly to hydrolysis, biodegradation, photolysis and evaporation. Each of these mechanisms will depend upon the physicochemical properties of the pesticide and the water matrix. Volatilization from the water is also important, and this is related to the water solubility (WS) and vapour pressure (VP) of the pesticide. When this mechanism is predominant, storage in the original water matrix using tightly stoppered bottles in the dark at 4°C might be the preferred method.

In this chapter, various methods of sample storage and preservation are discussed. These include standard methods such as the addition of acids, freeze-drying, the use of SPE disks and cartridges, and disposable SPE precolumns.

TABLE 2.5

ORGANOPHOSPHORUS PESTICIDES THAT HAVE BEEN WITHDRAWN FROM THE NATIONAL PESTICIDE SURVEY LIST BECAUSE THEY SUFFER LOSS WHEN STORED AT 4°C FOR 14 DAYS IN WELL WATER AFTER THE WATER HAS BEEN BIOLOGICALLY INHIBITED

Aspon	Malathion ^a
Ethyl-Parathion ^{a,b}	Disulfoton
Azinphos-methyl ^a	Methyl-parathion ^{a,b}
Famphur	Disulfoton sulfone
Fenitrothion ^{a,b}	Terbufos
Demeton ^a	Disulfoton sulfoxide
Fensulfothion	Phorate
Fenthion ^a	EPN ^c
Diazinon ^b	Phosmet
Diclofenthion	Ethion
Fonofos	

^aPesticides included in the 76/464/EEC Council Directive list of pesticides to be monitored in the aquatic environment.

^bStable at 4°C during 8 days at pH 8 in water from river Axios Greece, see Ref. [48].

^cO-Ethyl O-4-nitrophenylphenylphosphonothioate.

2.2.2. Standard methods of storage

The main factors affecting the stability of analytes in water are discussed in a recent review [54]. They include the character of the sample, the nature of the sample container, and the conditions of storage (temperature, darkness, use of preservatives, and time interval between sampling and analysis). A summary of the recommended standard preservation techniques was given in Table 2.2. Amber glass containers are generally preferred for collection when dealing with pesticides and organic compounds in general. The composition of the container caps which contact the sample or the extraction solvent can be a problem, and aluminium foil is used frequently to prevent contamination. Compounds such as phthalates or polydimethylsiloxanes usually cause problems because they are also found in rubber inserts or septa used with preservation vessels.

Some suggestions are given in a recent article [55] on the storage of water samples. First, after routine sampling, the sample containers should be exposed to negligible illumination. Secondly, the time between sampling and analysis can be divided into two periods: the first is immediately after sampling when the temperature of samples is not controlled; the second period is when the samples are placed in a refrigerator and the storage conditions are controlled. Thirdly, it should be assumed that the instability of samples increases with increasing temperature. Fourthly, the type of agitation envisaged can have some significance for the results, and stability tests should try to reproduce the transport of samples after collection.

Studies comparing various standard preservation methods have been performed and a few examples are reported below. Sixteen organophosphorus pesticides were preserved in distilled and creek water, using four preservation methods: the addition of chloroform, refrigeration, and maintenance at pH 4 or 7 [56]. Chloroform was the most effective preserving agent, by extracting the compounds and killing the organisms in the water. Chloroacetanilide herbicides such as alachlor, metolachlor, and propanil, and two phenoxy acid herbicides, 2,4-D and dichlorprop, showed stability when spiked in natural ground water at 15 and 22°C. Losses were important for propanil only after 8 months whereas the remaining four herbicides exhibited little or no degradation at a concentration of 1 µg/l, even after 12 months [54]. Forty-eight pesticides from the river Axios were stored at 4°C for 8 days using natural river water. After this period, phenmediphan was lost, and the recoveries of other pesticides, aldicarb sulfone, oxamyl, and methomyl, were slightly affected [48]. These results agreed with previous studies on carbamate stability [53]. Since the addition of preservatives into the water may damage some of the compounds during storage, an interesting comparison was made between the using HgCl₂ (as recommended by the US EPA), with the bottles of water in the refrigerator during 21 days, as opposed to using the same bottles without a preservative. The use of HgCl₂ had negative effects on compounds such as methyl azinphos, chlorpyrifos, dimethoate, disulfoton, fenitrothion, fenthion, malathion, metamitron, parathion, phoxim, pirimiphos, propyzamide, and vamidothion [58]. Some of these controversial results can be compared to those of the US EPA (see also Table 2.5) and we can conclude that HgCl₂ should be limited as a preservation agent to those cases where previous investigation had shown that there was no damage to real samples. These examples indicate that there is no universal rule to prevent changes in water samples, and that so-called standard or conventional methods are not always appropriate.

Finally, we should consider as a standard method the storage of analytes as sample extracts [59]. If immediate analysis is not possible the addition of the extraction solvent and storage at 4°C may be appropriate [54]. The US EPA conducted a study on the long-term chemical stability of organophosphorus compounds (OP) [59]. Fifteen OP were stored in four commonly used organic solvents (methanol, acetonitrile, dichloromethane, and acetone–dichloromethane) and at two concentration levels, 10 and 100 µg/ml. These solutions were stored in the dark between –15 and –20°C in screw-capped vials specially designed to minimize solvent evaporation. During this study, various analytical problems were detected, e.g., on-column degradation of trichlorfon and Naled to dichlorvos, and no conclusion could be drawn about their stability. In general, it was observed that the extracts are much more stable than the water samples, and that for organochlorine pesticides the extracts can be stored up to 40 days, whereas the water samples can only be stored for 7 days (see Table 2.2). In general, stability problems were found for organophosphorus and carbamate pesticides in water, whereas chloroanilides, triazines and phenoxy acids are quite stable.

2.2.3. Freeze-drying

Other ways of stabilization have been reported in the literature, e.g., freeze-drying with the addition of glycine [60]. Glycine is added to the water samples prior to freeze-drying, and together with the spiked pesticides, because of its effectiveness as a protective substance during lyophilization and because it can act as a support for the disperse phase when the water is being eliminated. For a better calculation of the losses during stabilization a general expression has been used that assumes the samples stored at -20°C in the dark are stable and can be accepted as reference comparisons for the samples stored at room temperature over a period of 12 months. Table 2.6 gives the ratios (R_T) of the mean values of the three measurements made at different periods of time (3, 6 and 12 months) against the mean value obtained for three determinations made on samples stored at -20°C . The uncertainty (U_T) obtained from the coefficient of variation (CV) of each set of three measurements at the different periods of time (3, 6 and 12 months) was also calculated. This varied from 0.02 to 0.12.

$$U_T = (CV_T^2 + CV_{-20^{\circ}\text{C}}^2)^{1/2} R_T / 100$$

where U_T is the uncertainty, CV_T is the coefficient of variation at 3, 6 or 12 months, $CV_{-20^{\circ}\text{C}}$ is the coefficient of variation at -20°C , R_T is the ratio between the

TABLE 2.6

THE RATIOS (RT) OF THE MEAN VALUES OF THREE MEASUREMENTS MADE AT DIFFERENT PERIODS OF TIME (3, 6 AND 12 MONTHS) OF STORAGE OF FREEZE-DRIED WATER CONTAINING PESTICIDES AGAINST THE MEAN VALUE OBTAINED FOR 3 DETERMINATIONS MADE ON FREEZE-DRIED WATER SAMPLES STORED AT -20°C : (A) FREEZE-DRIED WATER CONTAINING PESTICIDES AT HIGH SPIKING LEVEL (11–100 ppb), AND (B) LOW SPIKING LEVEL (0.1–5 ppb)

Compound	A			B		
	3 months	6 months	12 months	3 months	6 months	12 months
Cyanazine	0.67	0.32	0.12	n.i.		
Simazine	1.02	0.98	0.98	0.60	0.35	0.20
Carbaryl	1.11	1.10	0.85	1.02	0.62	0.38
Atrazine	1.05	1.05	0.96	0.80	0.60	0.27
Fenitrothion	0.46	–	–	0.60		
Fenamiphos	0.75	0.66	0.54	0.90	0.76	0.48
Tetrachlorvinphos	–	–	–	–	–	n.i.
Parathion-ethyl	1.10	1.12	0.87	0.91	0.50	0.33
Propanil	n.i.	1.01	0.95	0.94		
Linuron	n.i.	0.97	0.85	0.85		

n.i., not investigated; –, complete degradation <0.01 .

TABLE 2.7
PHYSICOCHEMICAL PROPERTIES OF PESTICIDES (FROM REFS. [62–64])

Compound	Vapour pressure (2×10^{-7} Pa)	Water solubility (mg/l)
Cyanazine	2	170
Simazine	30	6
Carbaryl	3000	40
Atrazine	400	33
Fenitrothion	72000	30
Fenamiphos	1300	700
Tetrachlorvinphos	40000	11
Parathion-ethyl	6000	15
Propanil	36000	268
Linuron	14000	75
Alachlor	19000	240
Metolachlor	42000	530
Trifluralin	150000	0.3
Dichlorvos	70000000	10000
Fonofos	280000	17
Mevinphos	170000	600000
Phosmet	2000	20
Ethofumesate	6500	50
Phemediphan	0.01	5

mean values at 3, 6 and 12 months at room temperature versus the mean value at -20°C .

From Table 2.6 it can be seen that the compounds that exhibit good stability over the whole 12 months at 20°C were propanil and linuron. Other compounds stable for at least 1 month were carbaryl, atrazine, simazine, fenamiphos and parathion-ethyl. Stabilization of fenamiphos and parathion for 1 month was feasible but the system was not effective for fenitrothion and tetrachlorvinphos. More recently [61], a stability study using freeze-dried water without any glycine indicated that atrazine, carbaryl and propanil were stable over a period of 1.5 months at 4° and 20°C , whereas simazine and linuron suffered losses of 16–20% after this storage period. Fenamiphos suffered higher losses and only about 20% of the compound remained after 1.5 months of storage at 20°C .

The different behaviours of stored pesticides can be explained by their vapour pressure and water solubility. In Table 2.7 these two physicochemical parameters are shown for various pesticides [62–64]. The more stable compounds show low vapour pressure and medium water solubility (up to 40 mg/l). Poor stability can result from high water solubility, as is the case for cyanazine and fenamiphos, with values up to 700 mg/l, or from a relatively high vapour pressure, as for the organophosphorus pesticides fenitrothion and tetrachlorvinphos. Fenitrothion suffered higher degrada-

tion than parathion, even though both molecules are very similar (they differ in the methoxy/ethoxy group and the presence of an ortho methyl group). Two factors are responsible for the higher stability of parathion: first, ethoxy groups are more stable than methoxy groups, and the presence of an ortho methyl group causes the molecule of fenitrothion to be more affected by photodegradation [52].

The preceding results are in agreement with literature reports on the degradation of pesticides under real environmental conditions. Short half-life values, of less than 1 day, were reported for fenitrothion [4,65], whereas for cyanazine, half-lives of 15 days were reported (much lower than for other triazines, e.g., atrazine with a value of 60 days) [64]. These results also match those from more stable compounds, since only ca. 2% of loss has been estimated for atrazine and simazine under real environmental conditions during a period of 20 days. Cyanazine has also been found to be much less stable than atrazine and simazine in surface waters of the USA; this has been attributed to oxidation of the cyanazine group [66].

In conclusion, it should be noticed that the lyophilized powder stored at -20°C showed no degradation or losses for any of the pesticides studied. When stored at either room temperature or 4°C , degradation problems were noticed. Only propanil and linuron from the various pesticides reported were stable over a period of 6 months when stored at room temperature [60,61]. In general, the storage of the different pesticides after freeze-drying depends on their physicochemical properties.

2.2.4. SPE disks

The use of solid-phase extraction (SPE) provides an alternative to storage of the original matrix. Early studies showed that fenitrothion was preconcentrated on an XAD-2 column and the samples remained stable for 5 weeks at room temperature [67]. Recent stability studies have been carried out using Empore disks [47,68–71]. The results indicate that pesticides have equivalent or greater stability on SPE disks than when stored in water at 4°C . Several pesticide groups were studied using this approach. Tomkins et al. [68] reported on the stability of DDE, DDT, dieldrin, endrin, aldrin, isodrin, α -chlordane and γ -chlordane in the disks over 4 weeks. Johnson et al. [70] obtained good stability data for pesticides such as 2,4-D, triclopyr, molinate, carbofuran, and thiobencarb stored for 180 days at -20°C . After this time, alachlor, methyl parathion, metolachlor, pendimethalin, norflurazon and proflufenos showed good stability whereas trifluralin exhibited 40% loss after preconcentrating waters containing $20\text{ }\mu\text{g/l}$ each. Captan [69] and carbofuran [70] were the least stable of the pesticides evaluated, with losses ranging from 13 to 100% depending on the length of storage. One of the reasons reported for such degradation was attributed to hydrolysis in the disk by the remaining water that had been removed by vacuum filtration.

From the examples reported above, it seems clear that the use of Empore disk material gives better results than storing water samples under conventional condi-

TABLE 2.8

COMPARISON BETWEEN THE RATIOS OF THE MEAN VALUES OF THE THREE MEASUREMENTS ON SAMPLES STORED FOR 1 AND 3 MONTHS AT ROOM TEMPERATURE IN FREEZE-DRIED WATER AND IN EMPORE DISKS, AGAINST THE MEAN VALUE OBTAINED FOR THREE MEASUREMENTS MADE ON SAMPLES STORED AT -20°C . INDIVIDUAL CONCENTRATIONS OF STORED PESTICIDES VARIED BETWEEN 0.6 AND 1.9 FREEZE-DRIED WATER) AND 0.6–1.9 AND $3\text{ }\mu\text{g/l}$ (EMPORE DISKS) (FROM REF. [71])

Compound	Freeze-drying		Empore disks	
	1 month	3 months	1 months	3 months
Atrazine	0.80	0.60	0.90	0.92
Simazine	0.60	0.35	0.96	1.00

tions (see Table 2.2). Examples of SPE storage compared to freeze-drying were also reported for atrazine and simazine [71]. A comparison between the SPE disk and freeze-drying stability data for water spiked with pesticides is presented in Table 2.8. The ratios of the mean values of the three measurements on samples stored for 1 and 3 months (at room temperature) against the mean values of the sample stored at -20°C are reported. The use of Empore disks is better than freeze-drying of the water sample since less alteration of the compounds of interest takes place and there is better stability of the sample. In order to improve the stability of pesticides on Empore disks, a comparative study was undertaken of different desiccation methods applied to pesticide enriched on SPE disks prior to storage [72]. After enrichment, the disks were treated by freeze-drying, vacuum desiccation with CaSO_4 , storage in direct contact with CaSO_4 , or without desiccation. The disks were stored for 3, 10 or 30 days at either ambient or freezing temperatures. The various desiccation methods were equally effective at removing water from the disks. However, it was postulated that freeze-drying removed water faster than the other methods, because of the vacuum conditions, and gave higher recoveries of analytes susceptible to hydrolysis, such as propanil and methyl parathion. It was reported that simazine, atrazine, alachlor and metolachlor were not affected by desiccation, duration of storage, or storage temperature. This is somewhat at variance with the results of the previously mentioned paper [71] that showed losses up to 24% of alachlor stored at room temperature. The major differences between these two experiments are the use of natural estuarine water in Ref. [71] instead of deionized water in Ref. [72], and the fact that a desiccation treatment with CaSO_4 was introduced in Ref. [72] but not in Ref. [71].

In general, it can be concluded that stabilization in Empore disks is both feasible and to be recommended. Various treatments can be performed, such as freeze-drying, to remove water faster and stabilize analytes such as propanil and methyl parathion that can easily hydrolyze. Trifluralin could not be stabilized, probably because of its relatively high vapour pressure (VP) compared to other analytes (see

Table 2.7). For other compounds such as carbofuran and captan, hydrolysis was the main cause of the degradation which occurred on the disk surface.

2.2.5. SPE cartridges

Very few papers have been published on the use of SPE cartridges for storage of organic pollutants preconcentrated from water samples. Fenitrothion was stabilized on an XAD-2 column for 5 weeks at room temperature [67]. Other papers [58,73,74] show a variety of results on the stability of priority pesticides on different kinds of cartridges such as polymers, C₁₈ and carbon.

Pesticides such as fenamiphos, phenmediphan and ethofumesate were stable on polymeric styrene-divinylbenzene cartridges, after preconcentration and storage at -20°C, 4°C and room temperature for 80 days [73]. Figure 2.3 shows the % recovery of the three priority pesticides stored on the SPE cartridge at 4° and -20°C. Firstly, it is apparent that phenmediphan and ethofumesate are stable during the whole storage period, as might be expected from their physicochemical properties (see Table 2.7). The results were a notable improvement on the losses of phenmediphan in water at pH 8 during 1 week at 4°C [8]. The compound fenamiphos suffered from hydrolysis and photochemical degradation. Even at -20°C it is unstable (after 80 days only 50% of the compound is recovered), whereas at 4°C only 30% of the parent compound remains after the same storage period. The formation of the metabolite, fenamiphos sulfoxide, was confirmed by using liquid chromatography-high flow pneumatically assisted electrospray-mass spectrometry. Such degradation follows the expectations from studies in the freeze-drying of water containing fenamiphos [60].

Two papers on the use of graphitized carbon black (GCB) for stabilizing phenylurea herbicides [74] and 34 selected pesticides [58] have been published. The herbicides were stable upon storage on this adsorbent at room temperature [74]. In the second study, it was shown that the best results were obtained by first minimizing the water content on the GCB extraction cartridges by a suitable methanol wash and then freezing the cartridges. Under these conditions, and over a storage period of 3 weeks, the stability of pesticides extracted from four river water samples onto the GCB surface was assessed. Twelve pesticides (carbaryl, dimethoate, disulfoton, ethiofencarb, fenitrothion, fenthion, malathion, metamitron, metribuzin, phoxim, primiphos and propyzamide) of the 34 pesticides were to a greater or lesser extent degraded upon storage at RT on the GCB. Many of the 12 pesticides, basically the organophosphorus and carbamates, were partly degraded by storing them in water and the rate of degradation was increased by storing them on GCB cartridges. This increase might be a result of hydrolysis reactions that can be catalyzed by adsorption on the graphitized carbon black surface. For metamitron, metribuzin, and dimethoate a considerable loss of the analyte was noticed. In this case it was postulated that particular adsorbates migrate slowly on the sorbent surface towards oxygenated sites contami-

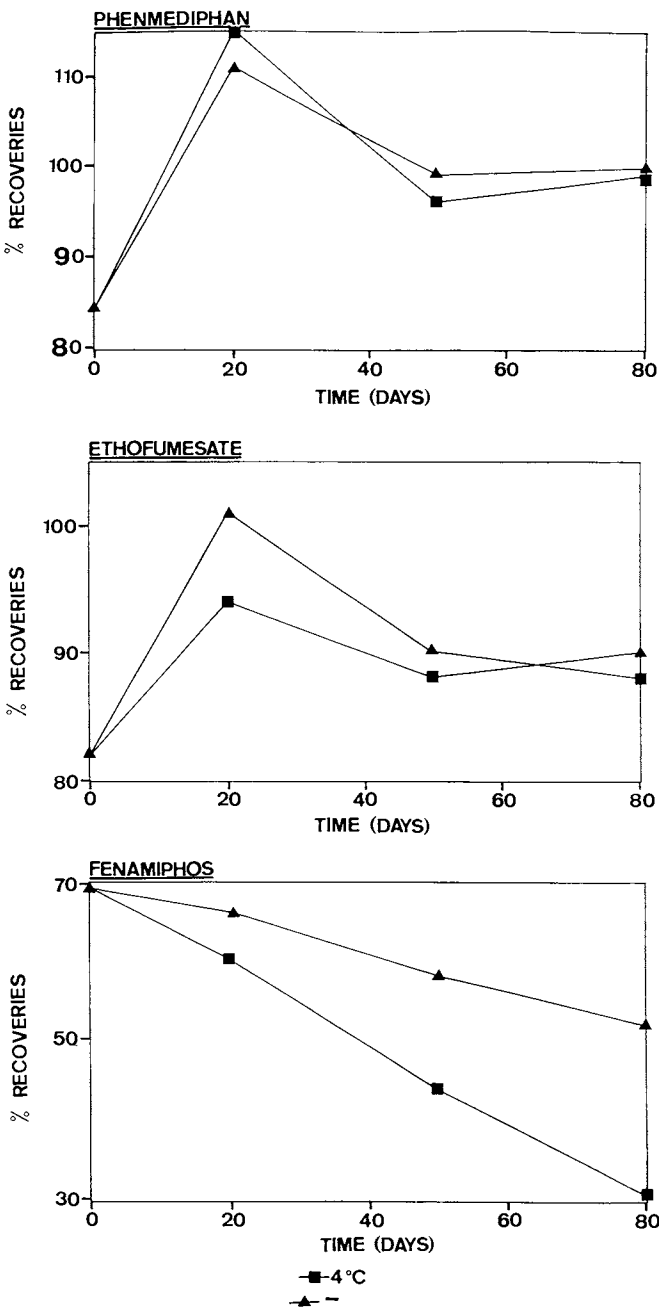


Fig. 2.3. Mean recovery (three replicates) of phemediphan, ethofuesmate and fenamiphos from SPE columns stored at 4°C and -20°C for periods up to 80 days.

nating the GCB surface, where chemisorption takes place. In summary, for the storage of pesticides on GCB it was concluded that three mechanisms of degradation occur: non-catalyzed hydrolysis by residual water; hydrolysis catalyzed by the GCB surface as a whole or by its surface chemical heterogeneity; and chemisorption caused by the same surface-active sites. In any case, storage at -20°C allowed all the pesticides studied to be stabilized without appreciable loss. More research is needed in this area, and the use of different cartridge materials and desiccation processes will be of help in such studies.

2.2.6. Disposable SPE precolumns

On-line disposable SPE precolumns are currently used with instruments such as the Prospekt coupled to liquid chromatography-diode array detection (LC-DAD). LC-DAD is currently used in the Rhine monitoring programme and other European programmes [49,75].

Table 2.9 shows the percentage recovery of each compound after storing the precolumns at 4°C for up to 6 weeks and for 8 months at -20°C . Figure 2.4 shows typical chromatograms before and after the various types of storage. The trend is that by the majority of the pesticides maintain a concentration between around 70 and 130% (with a deviation below 10%) over the whole time, indicating that degradation did not occur. Higher recoveries were found for temephos and chlorpyrifos, as a result of interferences in the chromatogram.

The stable compounds have a water solubility below 145 mg/l, a low vapour pressure, and K_{oc} above 236. At -20°C , out of 19 compounds analyzed, three underwent degradation (mevinphos, dichlorvos, and phosmet) while two compounds, fenamiphos and fonofos, totally degraded on the precolumns. The high solubility of mevinphos and dichlorvos accounts for possible hydrolytic processes on the C_{18} precolumns. Moreover, mevinphos has a K_{oc} of 44, which indicates a higher partitioning into the water phase than onto the C_{18} material. The very high VP for dichlorvos, and relatively high VP for mevinphos and phosmet, are also important for the observed degradation. Figure 2.5 shows the mean recoveries (three replicates) of mevinphos, phosmet, chlorfenvinphos and fonofos from precolumns stored at -20°C after 2, 2.5 and 8 months storage.

At 4°C , total elimination of fenamiphos occurred, reflecting its high solubility and instability in water (half-life 1.8 days) (see Table 2.9) in a similar way to that observed on freeze-drying [60] and when stored in conventional cartridges [73]. The disappearance of fonofos was unexpected since it is a quite apolar compound, with a half-life of 40 days. However, it has a vapour pressure of 28 mPa, much higher than other pesticides, and it is lost by evaporation.

It can be concluded that, as with freeze-drying or using other SPE materials, storage at -20°C provides the highest recoveries as hydrolysis or evaporation are diminished.

TABLE 2.9

MEAN RECOVERY (%) AND RELATIVE STANDARD DEVIATION (STD, $n = 3$) FOUND FOR PESTICIDES IN GROUND WATER USING ON-LINE SPE-LC-DAD, AND MEAN % RECOVERY ($n = 3$) FOR PESTICIDES STORED AT 4°C FOR 1.5 MONTHS AND DURING 8 MONTHS AT -20°C (ADAPTED FROM REF. [75])

Compound	ID. no.	Lambda	Mean recovery (% \pm STD)	Mean % recovery	
				1.5 months at 4°C	8 months at -20°C
Mevinphos- <i>cis</i>	1	220	106 \pm 0.8	32	22
Mevinphos- <i>trans</i>	2	220	102 \pm 1.0	45	58
Dichlorvos	3	220	106 \pm 1.4	117	55
Fensulfothion	4	254	104 \pm 1.1	101	89
Azinphos-methyl	5	220	98 \pm 0.15	115	111
Fenamiphos	6	254	101 \pm 2.4	n.f.	101
Phosmet	7	220	98 \pm 0.23	86	34
Pyridafenthion	8	254	101 \pm 0.6	106	100
Parathion-methyl	9	280	98 \pm 1.44	127	105
Malathion	10	220	101 \pm 1.5	88	124
Fenitrothion	11	254	99 \pm 2.08	110	108
Azinphos-ethyl	12	254	99 \pm 2.00	110	108
Chlorfenvinphos	13	254	100 \pm 1.6	110	106
Fenthion	14	254	101 \pm 1.6	93	103
Parathion-ethyl	15	280	100 \pm 0.7	114	109
Coumaphos	16	280	101 \pm 1.3	118	113
Fonofos	17	254	101 \pm 1.8	n.f.	107
EPN	18	220	108 \pm 3.7	128	83
Chlorpyrifos	19	220	114 \pm 4.4	132	133
Temephos	20	254	111 \pm 9.2	148	130

Volume of water preconcentrated = 26 ml, spiked at 10 ng/ml. n.f., not found.

2.2.7. Conclusions

The use of SPE materials seems to be a good alternative for the storage of pesticides preconcentrated from water samples, and it should be developed further in the near future, with many more applications. As a rule, storage should be at -20°C, immediately after preconcentration of the pesticides from water. A great advantage of using SPE for stabilizing pesticides in water samples is the reduction in the storage space required, compared to 1 l bottles of water. The easy shipping of the SPE materials containing pesticides to a central laboratory for final analysis is another advantage, since it is not necessary to perform the analysis immediately after sampling.

Problems may occur for certain pesticides stored at 4°C, and for many of them at room temperature; this is especially true for some organophosphorus and carbamate

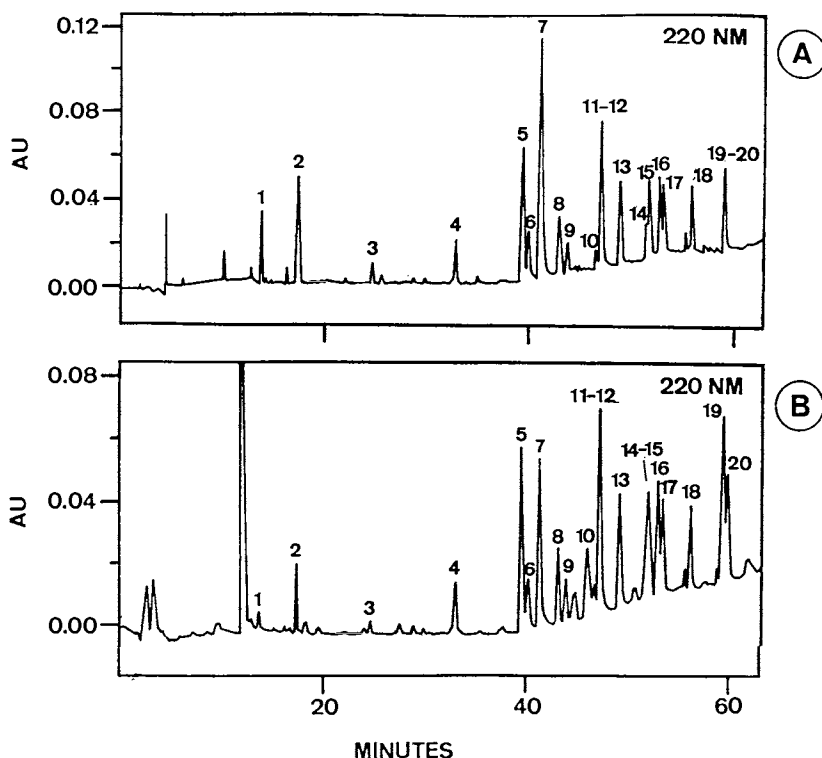


Fig. 2.4. On-line SPE LC-DAD chromatograms at 220 nm corresponding to (A) time = 0 of a ground water sample spiked with the pesticide mixture (for peak numbers see Table 2.9) at 10 ng/ml and (B) the same mixture after storage at -20°C for 8 months.

pesticides. The losses observed during the storage period are related to the physico-chemical properties of the pesticides, e.g., high vapour pressure or high water solubility, which favour losses by volatilization or hydrolysis. The type of SPE material is another factor to take into consideration. Problems arise from either the silanol groups present in the C_{18} material which favour hydrolysis by the water left after incomplete drying, or the chemical composition of the surface, such as occurs on GCB by catalyzing reactions or chemisorption.

Storage of pesticides on disposable SPE precolumns is a good alternative. Disposable SPE precolumns are very handy in carrying out monitoring studies, since they save time compared to off-line SPE procedures using either cartridges or disks. On-line SPE systems, such as SAMOS are currently being used for monitoring pesticides in the Rhine basin programme. Pesticides which were withdrawn from the NPS-EPA list, e.g., fenitrothion, fenthion, and parathion-methyl among others, were recovered when stored upon C_{18} precolumns. For these reasons, the SPE techniques are very useful in stabilizing pesticides and can be used for monitoring "hot spots" or

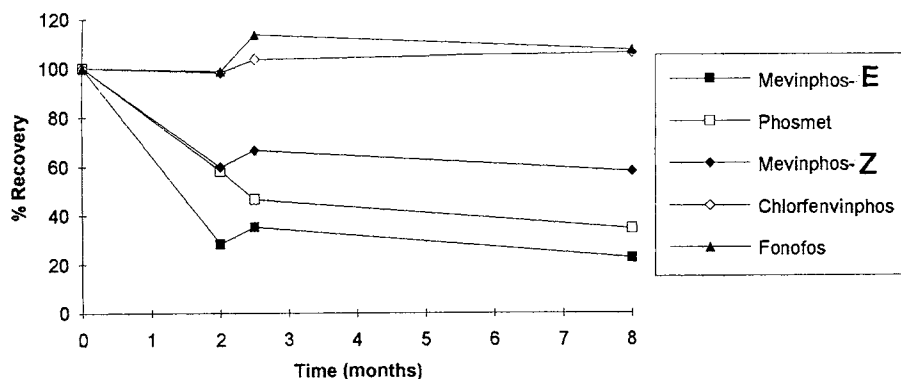


Fig. 2.5. Mean recovery (3 replicates) of mevinphos. (*E*) and (*Z*), phosmet, chlorfenvinphos and fonofos from precolumns stored for 2, 2.5 and 8 months at -20°C .

accidental spills. This will be the case with organophosphorus pesticides that are often sprayed in estuarine areas and where there is often a need to know the concentration of the analytes immediately after manual or aerial spraying. The immediate pre-concentration of the analytes in SPE cartridges or disks provides a good approach for rapid assessment of a spill.

From all the considerations mentioned, it is clear that research is still needed in this field and that the approaches reported here can be used for other pesticide families and other groups of pollutants that are usually preconcentrated with SPE materials from water matrices. Several points need to be considered however. More research is needed into the use of new polymeric sorbent materials with high specific surface areas, i.e., Lichrolut from Merck, to avoid the problems encountered with the more unstable pesticides such as fonofos and dichlorvos. Comparative studies are required, with and without pretreatment of the water samples by, for example, acidification, to improve stability. Water that remains in the cartridge material, even after drying, is still a problem. This is even more of a problem in the case of disposable SPE materials, since even the use of a freeze-drying step cannot ensure complete water removal. The use of polymeric sorbent materials should also avoid the problem of hydrolysis by the hydroxy groups. Stability studies for environmental work should be undertaken with the same water matrix as is being used in the on-going monitoring programme (ground, drinking and/or river water, etc.). There are no guidelines to follow for the stability of the analytes. Some analytes, e.g., triazines, are known to be stable on SPE materials, and for many other analytes a stability study should be undertaken. As a rule of thumb, scientists working in this field would be happy to observe stability at -20°C for 3 months, at 4°C for 1 month and at room temperature for 1 week. Knowing these basic time periods allows analysis of samples during a certain period to be accomplished. The 1 week period at room temperature usually permits shipping of samples by mail without any special requirements.

Certainly for any new pesticides, a study of the sort described is needed. Knowledge of the physicochemical properties of each pesticide should allow some guidelines for a stabilization protocol to be established in advance.

2.3. INTERLABORATORY PERFORMANCE STUDIES

2.3.1. Introduction

The increasing requirement to demonstrate the comparability of analytical data in environmental monitoring demands that there should be some external assessment of the quality of the results provided by individual laboratories. One way of doing this is by assessing the performance in interlaboratory comparisons, using centrally distributed samples [76]. Within the EU, the former Community Bureau of Reference (now “Measuring and Testing”) organizes a number of interlaboratory studies on environmental and other matrices. A review article [77] indicates the general objectives of intercomparisons, as follows. The first is to detect the pitfalls of a commonly applied method and to ascertain its performance in practice. The second is to measure the quality of a laboratory or a part of a laboratory, and then to improve this quality in collaborative work, as part of a mutual learning process. The ultimate purpose is to certify the contents of a reference material. In an ideal situation, where the results of all the laboratories are under control, only the last two types of intercomparison will be held. In practice, this equivalence of performance may take several years of participation in intercomparisons.

The aims indicated for intercomparisons will lead to three types of interlaboratory studies [1]: method performance studies, material certification studies and laboratory performance studies. These cover the main parameters, the analytical method used, the analyst in the laboratory and the material to be analyzed. The naming convention introduced by IUPAC in 1992 [78] indicates the following. In the method performance study, all the laboratories follow the same test method to measure a characteristic (usually a concentration of pesticide) in order to assess the performance parameter of the method. During the material certification study, a reference value is assigned to some characteristic in the test material, usually with a stated uncertainty. During the laboratory performance study, laboratories each use the method of their choice to measure a characteristic in order to assess the performance of the laboratory, and usually to evaluate or improve the performance. Use of vague terms such as “round robins”, “intercalibrations”, or “ring tests” are not recommended.

A questionnaire for interlaboratory studies has been published [79]. In this, several requirements are asked of the participating laboratories, such as (i) their willingness to participate in the precision experiment for the standard measurement method; (ii) that the participants will have all the standard apparatus and chemicals, and that the other essentials specified in the method will be available in the participant’s laboratory; (iii) that the “timing” requirements, such as the starting and finishing dates

will be followed; (iv) that the method will be strictly followed; (v) that samples will be handled according to the instructions; and (vi) that a qualified operator will perform the measurements. The questionnaire should be signed at the bottom, indicating that all the above-mentioned requirements will be met.

In practice, the objectives of interlaboratory studies can be mixed up. For example, in an improvement of the quality of measurement of an analyte (pesticide) in water the assessments of the method performance and the laboratory performance can be carried out at the same time. The objective is to improve both the method and the laboratory. In interlaboratory studies, identical samples are sent to each laboratory and the analytical results are sent back to the organizer who makes a statistical analysis of the data. A certified reference material is produced in the final phase of the project and this can be used to verify the accuracy of the analytical results. Reference materials can be used for the development of an analytical method and for day-to-day control. Cheap material, or laboratory reference material (LRM), will be used for day-to-day quality control. Expensive, high-quality certified material will be used less frequently.

In conclusion, interlaboratory tests are part of a laboratory's quality assurance programme whose objectives are to implement and increase the quality of analysis. Two major ideas are behind this objective: (i) that the programme should be understood as a learning programme, with a progressive enhancement in the analytical performance; and (ii) that learning through interlaboratory exercises is performed not only for the technicians but also for the teachers. The objectives of a quality assurance programme also include the statement of "teaching the teachers".

2.3.2. Aims and features of interlaboratory tests

The aims of interlaboratory tests for water quality control have recently been reviewed [1]. They include: (i) performing a collaborative study to test the capabilities of the analytical method, for instance to validate a candidate method for standardization; (ii) to gain a general picture of the errors existing in a group of laboratories. Usually this is performed using the Youden paired sample technique which provides a valuable means of summarizing the results of an interlaboratory test in graphical form; (iii) a special case uses the interlaboratory tests to arrive at a consensus (certified) value for the composition of a reference test material; (iv) to reach a consensus regarding a certified reference material (CRM). This has a more restricted scope than a reference material (RM) and basically requires a more rigorous choice of participants; (v) to perform proficiency tests to ensure that each group of laboratories achieves an acceptable standard and analytical accuracy, i.e., that the analytical errors are controlled within appropriately small bounds. This is the objective towards which most programmes of interlaboratory testing are directed, either explicitly or implicitly.

The term "proficiency testing" is used to indicate that the results generated in interlaboratory test comparisons are used for the purpose of a continuing assessment of

the technical competence of participating laboratories [80]. Alternative terms used for proficiency testing are “quality assessment” and “external quality assessment”. Proficiency testing is distinct from other interlaboratory tests, such as collaborative trials (that are used to establish a true value of an analyte concentration in a reference material). The aims of the proficiency testing are as follows: (i) to encourage good performance generally, and specifically to encourage the use of proper routine quality control measures within individual laboratories; (ii) to provide feedback to the laboratories and encourage remedial action where shortcomings in performance are detected; (iii) to provide a rational basis for removal of the selection or licensing of laboratories for a specific task should their performance fall below a certain standard. The motivation of these aims is clear; to identify laboratories that produce data of acceptable quality.

A successful proficiency test must provide certain types of information to the organizers. (i) It must enable a laboratory to compare its performance at a particular time with an appropriate external standard of performance. (ii) A given laboratory should also be able to compare its performance at a particular time with its performance in the past. (iii) It must enable the laboratory to compare its performance with that of other laboratories at a particular time. (iv) It must enable the organizers to identify participants whose performance is unsatisfactory. (v) To see whether there is a general improvement in performance with time.

As the complexity of interlaboratory tests is increased, it becomes possible to draw more conclusions concerning the sources and nature of errors which may be present. On the basis of this knowledge, it is then possible to direct efforts towards achieving the desired level of accuracy. In many cases, the interlaboratory exercises will serve as learning programmes, and only when all the participating laboratories perform such studies will the final measurements exhibit an acceptable precision. At a certain moment, all the participating laboratories will achieve an acceptable degree of performance; they will also have learned from previous mistakes, and better precision will be obtained. An example of improvements of proficiency testing is shown in Fig. 2.6, with the improvement in the between-laboratory agreement for the analysis of CB 118 in two similar fish oils in 1985 and, after a further learning programme in 1987. This study was organized by the BCR (now the Measuring and Testing Programme) of the Commission of European Communities.

Interlaboratory tests provides means of detecting and guarding against undiscovered sources of errors. In this respect, the three major sources of errors detected were classified [77] as being in: (i) the sample pretreatment, e.g., extraction, preconcentration, and separation; (ii) the final measurement, e.g., calibration errors, spectral interferences, or co-elution of peaks; (iii) the laboratory itself, e.g., the training and educational levels of researchers, technicians, and management.

Certain common issues must be considered in arriving at an appropriate design for an interlaboratory test. The organizers may consider the following points [1]. General considerations include providing general information to the participants, the se-

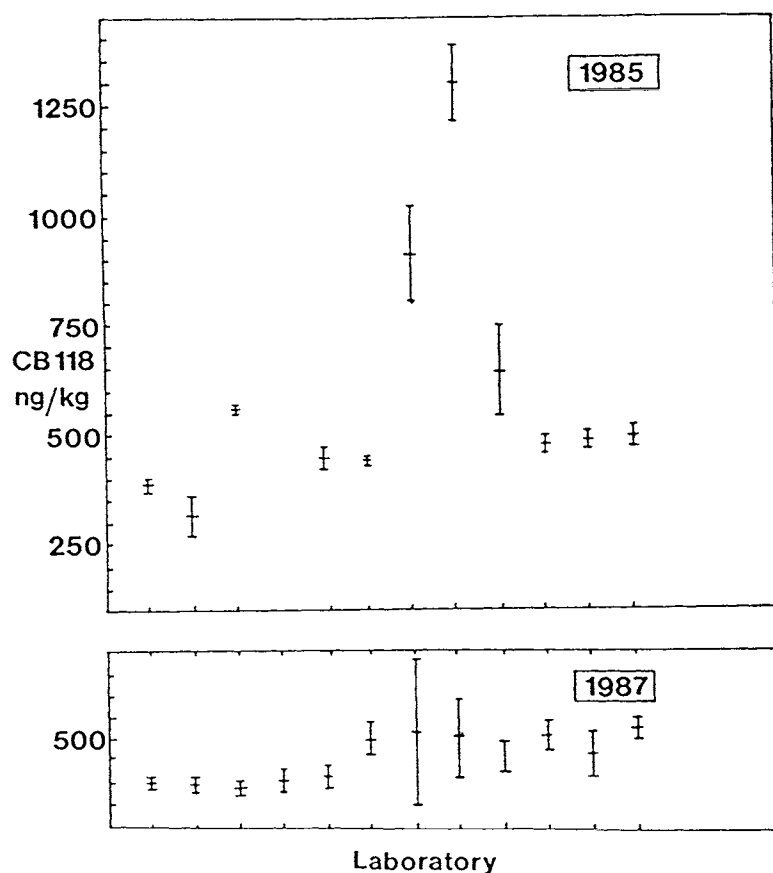


Fig. 2.6. The improvement in between-laboratory agreement for the analysis of CB 118 in two similar fish oils in 1995 (herring) and, after a further learning programme, in 1987 (mackerel). The data are given as laboratory means with $\pm 2SD$ confidence bar.

lection of the pesticides of interest, setting a timetable for analysis, and the number of participating laboratories. There is not always agreement about the number. For example, the IUPAC harmonized protocol has some strict rules such as the number of participating laboratories should be a minimum of eight, although it is mentioned that studies with more than 15 laboratories are unlikely [76]. The BCR indicates that having more than 25 participants in interlaboratory studies is difficult from the point of view of data evaluation [81]. Decisions have to be made regarding the test sample, e.g., the type and number of samples to be distributed, and the range of concentrations. Regarding the sample preparation, the organizer of interlaboratory exercises should provide information on the sample's homogeneity and stability and on how it should be stored. To clarify the analysis and reporting, the organizer should specify the information required to the laboratories, either if an analytical

method is previously selected or if the laboratories are free to use any method. Requirements about the use of one or two chromatographic columns (for confirmation purposes) and the number of replicate analyses should also be given by the organizer, as should information about the test evaluation, i.e., whether the organizer will supply special sheets to report the data, and how the data should be presented, with units, calibration and recovery values.

All the above information should be clarified in advance, prior to the performance of the interlaboratory test. The participating laboratories then have the basic information to participate in the interlaboratory test, and further discussions, when the test is performed, will be more valuable and will lead to surer conclusions about the reference material studied. Preliminary discussions before the interlaboratory study represent a key issue. It is important that all participants in an interlaboratory exercise should have set interlaboratory quality assurance and quality control systems. This implies that the method used for analysis is under statistical control in each participating laboratory.

2.3.3. Precision in interlaboratory tests

A point of controversy is the precision of the method. Before discussing the precision for pesticide analysis in water, we comment on the various terms that are used. In statistical practice, where the true value of a standard deviation, σ , is not known then it is generally replaced by an estimate, s , based upon a sample. In this respect, the terms used are generally the standard deviation of repeatability s_r , and standard deviation (SD) of reproducibility, s_R . Repeatability is the standard deviation obtained under identical circumstances affecting the laboratory, operator, equipment, and time interval, whereas reproducibility is the standard deviation obtained under different circumstances affecting the laboratory, operator, equipment, and time interval. In interlaboratory studies, the estimate of the reproducibility variance takes into account the repeatability variance within the laboratories taking part in the accuracy experiment which remain after the removal of outliers, and the estimate of the between-laboratory variance, s_L .

The relationship between the above-mentioned standard deviations is

$$(s_R)^2 = (s_r)^2 + (s_L)^2$$

The precision estimates are generally to be calculated both with no outliers removed and with outliers removed, using the Cochran and Grubbs outlier test. The Grubbs test should be applied to laboratory means and not to individual values or replicated designs [76]. The precision can be indicated in different ways, such as the repeatability or reproducibility coefficient of variation (CV), when it is indicated as a percentage with respect to the laboratory mean, m . Repeatability and reproducibility limits have been obtained by considering a 95% probability criterion and are ob-

tained by multiplying s_R or s_r by 2.8. This criterion means that the values for the differences between results obtained from the analysis of a sample would not be expected to be greater than the values for within- or between-laboratory determinations in more than 5% of cases [76].

Often, the term “bias” is used to indicate systematic errors when there is a persistent tendency for results to be greater or smaller than the true value (where the results are generally subject to positive and negative biases, respectively). As the systematic error of the bias of results is reduced, the trueness is said to increase. The major causes of systematic errors are: the instability of samples between sample collection and analysis; interferences; biased calibration; and biased blanks.

The criteria for outlier removal in interlaboratory exercises should be known in advance. In principle, outlier removal should be stopped when more than 22% (i.e., more than two out of nine laboratories) would be removed as a result of the sequential application of the outlier test [76]. This is not the criterion used by other bodies, e.g., Aquachek (Medmenham, UK) that organizes inter-laboratory exercises with water samples containing pesticides in Europe. Their target value for bias and precision is usually set at 17% [82,83]. This value of 17% is quite optimistic, since it corresponds to spiked pesticide samples at the ng/l level in ground water and waste water matrices. In contrast, recent studies organized by BCR [84] fixed a target for bias and for precision at 12% for chlorobiphenyls (CBs) in standard solutions and 25% for CBs in fish oil.

Many target values have been set prior to data analysis. Some scientists do not agree with this fixed measure of precision and it has been recommended that setting the precision should come after various inter-laboratory exercises. In the paper of Wells and De Boer [84] no fixed limits were selected, but the state of the art was reflected. In the final exercise, with different expert laboratories working in the field of marine chemistry, the between-laboratory CV of the standard analytes in iso-octane was 13–17% and that of the samples was 17–37%. These values are higher than those set in advance, and the sources of error were carefully evaluated. Over 60% of the errors resulted from inaccurate calibration and poor GC separation.

Horwitz et al. [85,86] found that the order of magnitude of uncertainties in an interlaboratory study is related to the concentration level. As a rule-of-thumb, as a first estimate, he found that many results in intercomparisons with trained laboratories followed the relationship

$$CV (\%) = 2^{(1 - 0.5 \log C)}$$

in which CV is the coefficient of variation and C the concentration of the analyte. Although this rule of Horwitz is not a law of nature, when participating laboratories have not been in quality exercises before, the results are most likely to be worse than indicated; well trained laboratories will do better.

In a recent book [87], it was indicated that published data and analytical experi-

ence reveal an interlaboratory basic variability of 50% (95% confidence level) for the analysis of pesticides in water matrices. These data were supported by a variety of studies with various groups of pesticides and techniques and covering a wide range of concentrations (from 0.04 to 5000 $\mu\text{g/l}$). The US EPA, through its National Pesticide Survey programme in Drinking Water Wells, and the Ciba-Geigy Laboratories at Basle, compiled many determinations of various pesticide groups and metabolites by either GC or HPLC methods. Taking into consideration the facts that, generally, the CV for trained laboratories in pesticide analysis residues in water varies between 12 and 14%, and that in the various interlaboratory exercises organized mainly in the USA, the random error varied between 7 and 23%, if outliers were not considered, it was proposed to adopt the so-called bracket values (b.v.) including 95% of all results [87]:

b.v. = mean values $\pm 2(\text{CV})$

Since the maximum CV of the results was 23%, the “basic variability” of pesticide trace analysis in water is $\pm 50\%$, expressed as a bracket value. This means, in practice, that all the results in interlaboratory studies of pesticides are well within 50% and 150%. If this term were accepted, then it means that the basic variability is 50%, the corresponding CV is 25% (inter-laboratory), and the intra-laboratory CV is about 15%. This is in good agreement with the data reported from the literature and the practical performance of every-day analysis of pesticides in water samples. It seems that the 25% interlaboratory values for CV is quite common to various programmes [82–84] and this seems to be a value to be taken into consideration for interlaboratory exercises of measurements of pesticides in water.

Another term frequently used for the assessment of the performance of laboratories is the z -score.

$$z = (x - X)/s$$

where x is the robust laboratory mean, X the assigned value, and s the standard deviation.

When X is the overall (robust) mean, s is based on the data, and the distribution is the standard normal distribution, then for 5% of the results $|z| > 2$; the probability is that for 0.3% of the results $|z| > 3$, and 95% of the results will exhibit $|z| < 2$. Therefore, it should be reasonable to state that z -scores are satisfactory when $|z| < 2$, questionable when $2 < |z| < 3$, and unsatisfactory when $|z| > 3$. In some cases, the assigned value is known beforehand. When the assigned value is unknown, it can be estimated by a critical evaluation of the results of some expert laboratories. An average estimate CV of 12.5% is commonly applied to many determinants common to water matrices, such as organochlorinated pesticides, PCBs, and PAHs. When analyzing standard samples such as atrazine, the Aquacheck program has accepted a good per-

formance to be when the overall CV is 5%. An example of z-scores, represented as % of bias, for atrazine is given in Fig. 2.7. This exercise was performed by Aquachek and the participating laboratories were expert in the analysis of triazines. Since usually in the Aquachek exercise many errors could be attributed to the extraction step, it was decided to analyze samples by direct injection, using either GC-MS or HPLC. We can note that three laboratories gave extremely high % of CV (54–99%), six laboratories gave CV between 17 and 31%, whereas the rest gave CV below 12.5%. Only seven results (using GC-MS) and three (using HPLC), out of 23 had CV below 5% , which was the accepted value considered by Aquachek. From the reported results, and the considerations given in the previous section, it seems clear that the estimated CV of 5% (for standard solutions) can be somewhat restrictive, and only excellent laboratories can achieve such target values. The acceptance of a CV of 12.5% for standard solutions and 25% for the determination of polar pesticides in water seems more realistic and reflects the present state of the art [84,87]. Using the more realistic CV of 12.5%, 14 laboratories out of 23 will have accepted bias in the results reported in Fig. 2.7.

Very few examples of interlaboratory exercises for the common pesticides used nowadays have been reported in the literature. Although many exercises were organized in the BCR-CEC for PCBs (see Fig. 2.6), PAHs, and organochlorinated pesticides, in various matrices, such as soil, fish oil, and waste, very few interlaboratory exercises have been organized for pesticides (organophosphorus, triazines, pheny-

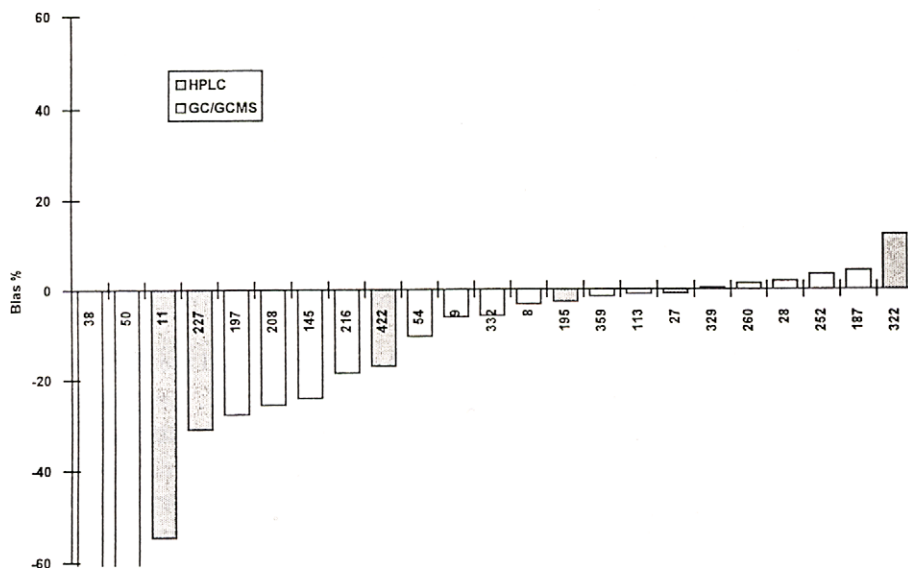


Fig. 2.7. Bias % plots for atrazine in water samples (Aquachek programme) when analyzing atrazine solution without a preconcentration step (direct injection into GC-MS or HPLC). The laboratory participation number is also indicated.

lureas) in water matrices. To our knowledge, only Aquachek in Europe is regularly organizing such exercises, with various groups of pesticides (organophosphorus, triazines, phenylurea, and acidic) spiked in water matrices. The BCR has organized two interlaboratory exercises with pesticides spiked in freeze-dried water. In the USA, various interlaboratory exercises for pesticides have been organized, mainly with standard solutions or highly concentrated samples. Other organizations, such as the International Atomic Energy Agency (IAEA) have also organized restricted interlaboratory exercises for pesticides among laboratories of the Mediterranean region and in some private companies, such as Ciba-Geigy also.

These examples are discussed below and show the major problems of organizing interlaboratory exercises with the common pesticides now in use. These problems are related to the degradation of these pesticides in water [7], resulting from the pH, temperature, and volatility, which are related to their stability in water and the water type used.

2.3.4. Freeze-dried water samples

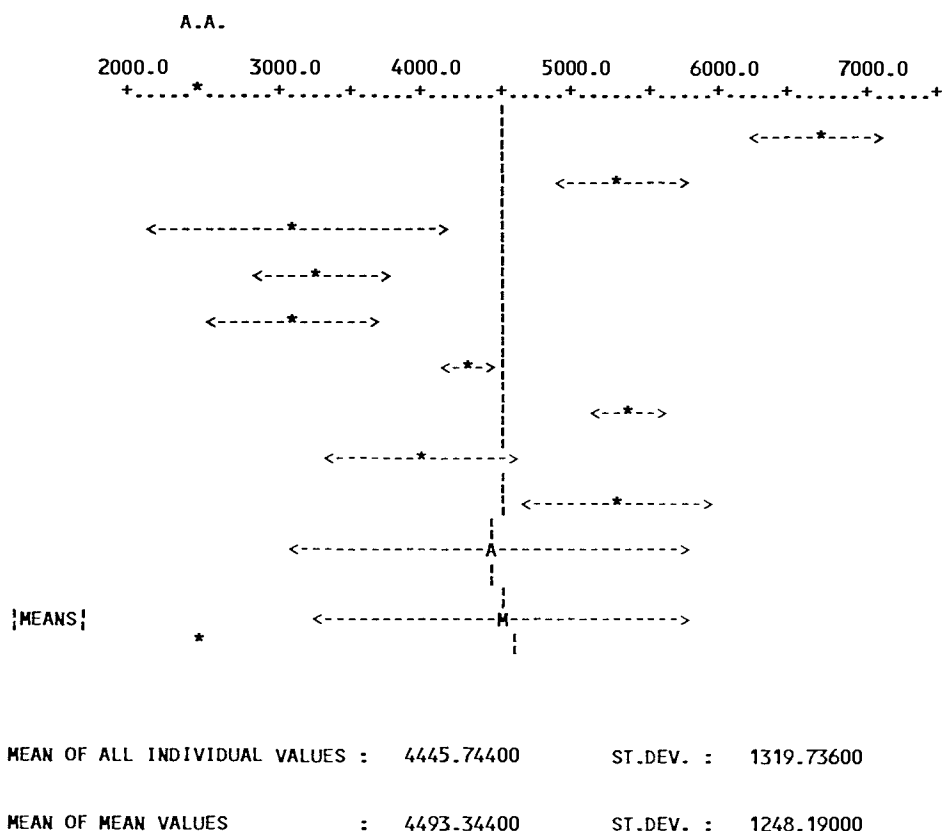
Before undertaking interlaboratory studies of water samples containing polar pesticides, the instability, preservation and transport of these samples should be investigated [7]. Two examples are reported in the literature that use freeze-dried water samples spiked with pesticides [60,61]. This material, after homogenization and stability studies, was used for a first interlaboratory exercise organized by the BCR (see Table 2.6). The guarantee that this material was stable in the freeze-dried water led the BCR to undertake the interlaboratory comparison. During a first meeting, prior to the interlaboratory exercise, the participants discussed the work to be done and the pesticides to be studied. Following the policy of BCR, each laboratory was asked to use its own method. This behaviour, generally in Europe, is based on the reasoning that the expert laboratory is more confident in its own methodology. This type of interlaboratory exercise approaches a proficiency testing or a laboratory performance study. As a result of this scheme, new methodology, such as automated on-line solid-phase extraction (SPE) followed by HPLC, was used by several laboratories.

Most of the participants were not familiar with the analysis of freeze-dried water samples and, as a consequence, a "tailor-made" procedure was given for the method of storage and reconstitution of the freeze-dried water. The participants were also asked to perform five independent determinations for reconstitution and to estimate the extraction efficiency and recovery of the method used (to be performed in triplicate), the determination for each compound of the linearity range, and also a procedure blank. The results and the description of the analytical procedures had to be reported using specially prepared reporting forms. These forms included quality control items which had to be checked; in particular in GC methods, it was requested that the identity of the peaks be verified by either a second column or by mass spectrometry. At least one internal standard had to be used for the quantification. Each

participant also needed to submit a chromatogram of the blank, the calibration solution, and the sample. Special consideration was devoted to the calibration step. The quality of the calibrants used had to be demonstrated, and when two different chromatographic methods were used (GC and HPLC) all the above-mentioned requirements had to be provided for each method.

The result of the first interlaboratory exercise using freeze-dried water was reported [60]. The discussion about the various pesticides is summarized below. For carbaryl the presence of glycine, added as a “keeper” in the water solution, did not affect its determination. Eight laboratories showed comparable results using either GC or HPLC methods. For atrazine, the presence of glycine affected the determination. The use of an organophosphorus compound as internal standard for the quantitation of atrazine and for simazine was shown to be inadequate for a NPD detection. Regarding simazine, some participants noticed that a low pH of the water reduced the extraction efficiency of simazine in LLE. Fenitrothion was unstable in the distributed samples, so no conclusions could be drawn. With parathion-ethyl, problems of volatilization were also noticed, and it could not be inter-compared. With fenamiphos a rather good comparability of results was noticed. For propanil, interferences were noticed by several participants but the overall results demonstrated good comparability, as shown in Fig. 2.8. With linuron, a poor performance was noticed and the participants concluded that the methods available for this compound needed to be revalidated in all laboratories.

In a second interlaboratory exercise [61], freeze-dried water spiked with pesticides, without the addition of glycine was used. Table 2.10 gives the mean concentration for each compound in three successive determinations in one of the laboratories, and the mean for all 20 participating laboratories. The participating laboratories used either GC or HPLC techniques, in about equal numbers. Most of the laboratories used off-line SPE techniques followed by either GC or HPLC methods. Only three laboratories of the 20 used on-line solid-phase extraction followed by LC, with either UV or diode-array detection. All concentrations were between the expected values given by the intercalibration study. Problems were encountered by many laboratories with the quantification of fenamiphos. As shown [9], fenamiphos is rapidly degraded in water. This is different when glycine is used as a preservative (“keeper”) in the water matrix; then, fenamiphos was stable and gave no problems. Most probably in this case, freeze-drying of water with no glycine, the behaviour of fenamiphos is similar to that observed in the water matrix whereas glycine prevents degradation [60]. Furthermore, there was a problem relating to the low concentration of this compound in the sample (see Table 2.10). Such a low concentration implies having more interferences when the quantitation is performed. The on-line SPE methodology permitted a rapid analysis between the concentration step and the LC analysis and so no problems of degradation of the fenamiphos were encountered in this case. Nevertheless, problems showed up due to its low concentration in the lyophilized sample.



A.A.: added amount

Fig. 2.8. Bar graph presentation of the results obtained in the interlaboratory study of propanil in one of the batches of freeze-dried water. Laboratories 01, 05, 15 and 16 reported the presence of interfering peaks with propanil.

The reported values in Table 2.10 fell within 25% of the coefficient of variation, so the exercise was satisfactory. These data [61] achieved a better precision between all the laboratories than did the previous interlaboratory exercise using freeze-dried water spiked with pesticides [60]. This is mainly attributed to two facts. In the first exercise, glycine was added as a preservative for the freeze-drying, and interferences in the determination of many freeze-dried pesticides were noticed. Secondly, the spiking level in the first intercomparison exercise was at 0.03–1.0 $\mu\text{g/kg}$ (one order of magnitude lower), so interferences and problems in the final determinations were higher. The standard deviations in the interlaboratory exercise for propanil, atrazine and fenamiphos were 27, 54, and 69%, respectively [60], which is within the range of acceptable values following the considerations reported in Section 2.3.3.

TABLE 2.10
MEAN CONCENTRATIONS OF SELECTED PESTICIDES AND THE MEAN CONCENTRATION
FOR ALL LABORATORIES [61]

Compound	Concentration ($\mu\text{g/kg}$)	
	Mean \pm SD	Mean \pm SD
Simazine	20.57 ± 0.53	17.3 ± 4.0
Atrazine	12.26 ± 0.28	11.4 ± 1.5
Carbaryl	6.25 ± 0.14	9.4 ± 2.4
Propanil	12.15 ± 0.17	11.7 ± 1.3
Linuron	5.13 ± 0.22	5.5 ± 0.9
Fenamiphos	1.16 ± 0.01	1.2 ± 0.5

The concentrations are given in $\mu\text{g/g}$ of freeze-dried water (solid powder).

2.3.5. The Aquacheck programme

The Water Research Centre (WRC) at Medmenham, UK, organizes interlaboratory studies throughout Europe. This is the most currently used interlaboratory programme in Europe for water samples spiked with pesticides. The detailed protocol is described elsewhere [82,83] and consists of spiking a water sample with a solution that contained various pesticides at an unknown concentration at a level imposed by the organization ($50\ \mu\text{l}$ of the standard for each 500 ml of water). The sample is then analyzed using the method developed by each laboratory and the reported results are afterwards intercompared with those of the rest of the participants and with the real spiked values. The percentage error is given by the organizer at the end of exercise, where the results of the certified values are given together with the mean of all participating laboratories.

The on-line SPE-LC-DAD using the Prospekt was validated by participating in different interlaboratory exercises organized by Aquacheck. In order to provide a reliable statistical report on the results obtained by all participants, a minimum of eight laboratories participating in interlaboratory exercises is mandatory. In this exercise, the criterion was followed and the number of participants varied from eight to 14 (Table 2.11). The rest of the participants used gas chromatography (GC) with selective detectors such as nitrogen phosphorus (NPD), flame photometric detector (FPD), or a thermionic detector (TSD). The GC techniques are well known and have been widely used for the determination of organophosphorus pesticides in various matrices. However, limitations arise because GC is unable to analyze non-volatile and polar compounds, so time-consuming derivatization is necessary prior to the final chromatographic step.

Table 2.12 shows the results of three interlaboratory studies obtained by one laboratory using ground water samples, and the percentage of error compared to the certi-

TABLE 2.11

NUMBER OF LABORATORIES PRODUCING ACCEPTABLE, FLAGGED (17% ERROR) AND DOUBLE-FLAGGED (34% ERROR) RESULTS FROM INTERLABORATORY COMPARISONS, CORRESPONDING TO MARCH 1994 AND SEPTEMBER 1994

Compound	March 1994				September 1994			
	T	A	S	D	T	A	S	D
Azinphos-methyl	12	7	1	4	12	6	2	4
Dichlorvos	11	7	2	2	11	3	3	5
Fenitrothion	12	8	1	3	13	7	2	4
Malathion	11	3	5	3	13	6	4	3
Mevinphos-(Z)	8	4	2	2	8	5	1	2
Chlorfenvinfos	12	9	2	1	14	6	1	7
Diazinon	12	2	6	4	14	1	4	9
Azinphos-ethyl	11	7	0	4	10	5	2	3
Fenthion	11	3	4	4	10	1	5	4
Parathion-ethyl	11	4	3	4	13	8	3	2
Parathion-methyl	10	4	3	3	10	5	2	3

T, total number of participating laboratories; A, laboratories giving acceptable results; S, laboratories giving single flagged results; D, laboratories giving double flagged results.

fied value from Aquacheck. The results are evaluated according to the limits imposed by the organization; results below 17% are acceptable. Flagged and double-

TABLE 2.12

MEAN CONCENTRATION (ng/l AND % OF MEAN DIFFERENCE ($N = 4$) IN RELATION TO REFERENCE VALUES OF ORGANOPHOSPHORUS PESTICIDES FROM THREE INTERLABORATORY STUDIES (RESULTS WERE OBTAINED FROM SPIKING GROUND WATER WITH THE CERTIFIED MATERIAL FROM AQUACHECK [83])

Compound	July 1993		November 1993		March 1994	
	ng/l	% error	ng/l	% error	ng/l	% error
Azinphos-methyl	98.3	23	24.4	32	97.8	6
Dichlorvos	46.0	36	63.9	21	54.9	69
Fenitrothion	34.0	17	23.4	9	47.3	14
Malathion	154	89	24.9	55	94.2	24
Mevinphos-(Z)-	n.r.	n.r.	64.6	0.9	74.3	4
Chlorfenvinfos	47.7	36	37.0	11	31.4	22
Azinphos-ethyl	95.2	12	41.9	24	100	12
Fenthion	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Parathion-ethyl	n.r.	n.r.	111	3.7	78.0	6
Parathion-methyl	26.0	22	97.4	15	69.7	24

n.r., not reported or unable to quantify.

flagged results are those which exceed the maximum acceptable error, or twice the maximum acceptable error. If we consider the AOAC limits, the maximum error between laboratories is 22%. As stated in the introduction of this section, 25% seems a more reasonable value for the precision in interlaboratory studies involving water samples spiked with pesticides. The reason is that Aquacheck interlaboratory exercises have the objective of improving and controlling the quality of water analysis. The target for CV, and for the precision set at 17% by Aquacheck, is quite strict since this limit cannot be established for the analysis of water samples spiked with pesticides. Owing to the difficulties of analyzing polar pesticides in water, the coefficient of variation and target values should be higher. As an example, the EEC-BCR certificate exercises for PCBs, an easier interlaboratory exercise, has established a precision of 12% in standard solutions and 25% in spiked samples [84]. Among these values, for the different participating laboratories which analyzed PCBs in spiked samples the CV ranged from 17.6 to 37%. This is a more realistic approach than the 17% fixed by Aquacheck.

The results obtained in the intercalibration studies show that 19 determinations are below 25%, six are above than 25%, and eight are not determined, mainly because of matrix interference problems in the diode-array traces. For dichlorvos, the cause of error is its poor UV absorbance and the fact that this compound is very polar, so that it elutes at the beginning of the chromatogram where matrix interferences are more abundant. The poor UV absorbance accounts for the quantification error of malathion. The quantification error of diazinon originated in its co-elution with fenthion. The second derivative revealed the presence of both compounds in a single peak. Moreover, peak purity analysis confirmed the non-homogeneity of the peak. Because of the high similarity between the two spectra it was impossible to discern both compounds through wavelength selection. The deconvolution and quantification of such compounds can be accomplished by a chemometric approach [88].

It was interesting to detect the presence of the two isomers of mevinphos [82,83]. Both of them could be identified in two of the three interlaboratory exercises using ground water samples, in contrast to GC techniques. The error encountered for mevinphos-(Z) in the exercises of November 1993 and March 1994 was only 0.9 and 4%, using ground water samples, which leads us to the conclusion that the isomer quantified and reported by the organizers was mevinphos-(Z), which could be quantified effectively using LC-DAD.

In a later stage [89], the use of on-line SPE followed by LC-atmospheric pressure chemical ionization (APCI)-mass spectrometry (MS) was used in the Aquacheck interlaboratory programme. The results obtained in one of the inter-laboratory exercises are reported in Table 2.13. It can be pointed out that in the PI mode most of the compounds gave acceptable values, below the 22% accepted by AOAC, with the exception of parathion-ethyl and fenthion. This can be attributed to the poor limits of quantitation of parathion-ethyl and fenthion being 37 and 25 ng/l, respectively. Since the certified amounts to be determined in the water were 25 and 55.5 ng/l for para-

TABLE 2.13

CONCENTRATION (ng/l) AND % ERROR RELATIVE TO REFERENCE VALUES AFTER PRECONCENTRATING 100 ml OF DRINKING WATER SAMPLE SPIKED WITH THE CERTIFIED SOLUTION FROM AQUACHECK

Compound	Positive ion		Negative ion	
	ng/l	% error	ng/l	% error
Mevinphos-(Z)	48.9	12.4	n.d.	n.d.
Dichlorvos	62.9	-2.2	n.d.	n.d.
Azinphos-methyl	50.9	2.4	31.5	-48.7
Parathion-methyl	60.2	-21.5	45.6	-29.6
Malathion	24.7	-10.2	73.3	9.2
Fenitrothion	48.2	-10.5	22.5	-18.4
Azinphos-ethyl	9.09	-0.5	62.0	28.1
Parathion-ethyl	38.8	-63.5	30.6	22.3
Fenthion	53.9	-30.0	n.d.	n.d.
Chlorfenvinphos	51.1	3.0	n.d.	n.d.

n.d., not determined [89].

thion-ethyl and fenthion, respectively, the LOQ are too close, or exceed the target values. By performing other experiments, it may be feasible to reach the target values. Since these two analytes exhibit breakthrough volumes above 300 ml, by pre-concentrating this water volume, the LOQs will increase by a factor of three and, in principle, better quantitation will be possible. However, when preconcentrating such a water volume, early breakthrough analytes such as dichlorvos and mevinphos-(E) and -(Z) will suffer poor recoveries and errors in their final determination. In addition, matrix interferences will also be enriched into the column, making the quantitation of various compounds more difficult.

Under NI mode, parathion-ethyl gave much better results than in PI mode because of its lower LOQ in NI mode, and consequently its determination was feasible. Fenthion and dichlorvos could not be determined, which can be attributed to their poor LOQ of 42–50 ng/l [89]. Considering that the target values were 50–55.5 ng/l in the ground water samples, and that the matrix was more complex than that used for the estimation of the LOQ, it is understandable that these two analytes were not determined. Fenthion exhibited additional problems because of its poor stability in water during the preconcentration step which caused degradation [82]. Some of the other compounds [mevinphos-(Z) and chlorfenvinphos] could not be determined by NI, which was mainly attributed to a matrix effect that enabled quantification using the m/z 125 ion.

In conclusion, we should state that the interlaboratory exercises organized by Aquachek are sensible and are the first step in interlaboratory exercises using water samples spiked with pesticide. To avoid degradation, the standard samples are sent

separately from the water matrix and the spiking should be carried out at the laboratory. The organization recommends that samples should be extracted immediately after the spiking is done, otherwise degradation of pesticides occurs. If the water samples with the pesticides already spiked were to be submitted, degradation problems and difficulties in interlaboratory exercises would certainly occur. The major question still remains, and refers to the transport of water samples containing pesticides, through interlaboratory exercises. We have partly asked this question in the first part of this chapter, by proposing methods of stabilization either using freeze-drying or SPE cartridges. This will probably be the trend in the coming years, since it is inconvenient and difficult to perform interlaboratory exercises using water samples spiked with pesticides. Indeed, the actual proposal of the WRC fills a gap in the area of interlaboratory studies for analyzing pesticides in water samples and it is probably, at present, the only way to perform interlaboratory studies of pesticides in water samples.

2.3.6. US EPA interlaboratory studies

The US EPA has organized interlaboratory studies within American laboratories, generally for compounds such as PCBs [90]. So far, the EPA have also been involved in giving credit to different laboratories and commercial firms through cooperative research and development agreements (CRADA) for the so-called “EPA-certified” materials [91]. The EPA has funded a programme involving various reference materials, from metals to organics. It appears that in the near future not all the groups of contaminants will receive the same attention. Metal standards are already of high quality and widely available, whereas for pesticides, this is less true. Probably, much more attention will be devoted to polar pesticides in the near future [91]. The most relevant interlaboratory exercises organized by the US EPA on polar pesticides and using GC, HPLC and even LC-MS are reported in Refs. [92–97], and are discussed briefly.

There are minimum quality requirements for participating in the interlaboratory exercises of the US EPA [93]. (i) There has to be an initial demonstration of laboratory capability. (ii) Analysis of surrogate standards has to be done as continuous checking on sample preparation, with acceptable recoveries of 70–130%. (iii) There is monitoring of internal standards as a continuous checking of system performance. (iv) There is analysis of a method blank with each set of extracts. (v) There is analysis of spiked reagent water as a continuous check with (vi) analysis of the daily instrument QC standard to ensure acceptable instrument performance, and (vii) there is analysis of an external unknown performance evaluation sample (when available) once a year.

The collaborative study of the US EPA method 515.1, “Determination of chlorinated acids in water by GC with an electron capture detector” was reported in Ref. [92]. The waters were spiked with 17 chlorinated pesticides (see Table 2.14), each at

TABLE 2.14

ACCEPTANCE LIMITS FOR THE ANALYTES OF A LABORATORY QUALITY CONTROL SAMPLE, AS PERCENT SPIKED VALUE % (US EPA METHOD 515.1, CHLORINATED ACID IN REAGENT WATER MATRIX)

Analyte	Spike value ($\mu\text{g/l}$)	Recovery	Overall SD	Acceptance limits
Bentazone	3	2.36	0.41	37–120
2,4-D	3	2.75	0.36	55–128
2,4-DB	40	39.7	6.34	52–147
3,5-Dichlorobenzoic acid	1	0.90	0.28	7–173
DCPA acid	2	1.66	0.34	32–134
Dicamba	1	0.98	0.09	70–126
Dichlorprop	4	3.90	0.54	57–138
5-Hydroxydicamba	1	0.99	0.29	11–187
Pentachlorophenol	1	0.87	0.18	34–140
Picloram	2	2.10	0.72	0–213
2,4,5-T	1	0.93	0.10	62–124
2,4,5-TP(Silvex)	3	2.93	0.41	57–139

The acceptance limits are %, defined as (mean recovery \pm 3 standard deviations/spike level. The spike level is 10–15 times the method detection limit (MDL).

six concentration levels. Eight laboratories extracted the spiked waters at $\text{pH} < 2$ with diethyl ether, performed a solvent exchange with methyl tert-butyl ether, prepared methyl esters of the extracted acids using diazomethane, and analyzed an aliquot of each extract by GC-electron capture detection (ECD). The mean percent recoveries at 10–15 times the method detection limit (MDL) ranged from 79 to 105% in reagent water and from 75 to 123% in finished drinking water. In reagent water, the overall precision and CV ranged from 11.9 to 37.0%. The acceptance limits for the analysis of a laboratory quality control sample are reported in Table 2.14. The method exhibited statistically significant matrix effects for five compounds: 2,4,-D, 3,5-dichlorobenzoic acid, dicamba, dichlorprop, and picloram. The recoveries above 100% resulted from inadequate correction for the background responses. Because of this, research on clean-up techniques was recommended and also that users of this method should routinely test a quality control sample prepared in reagent water and compare the results with the performance-based acceptance limits derived in this study (see Table 2.14).

Another collaborative study was undertaken for the determination of pesticides in finished drinking water, by HPLC with UV detection at 254 nm, according to the EPA National Pesticide Survey method 4 [93]. Ten volunteer laboratories extracted the water samples by dichloromethane liquid–liquid extraction, performed a solvent exchange with methanol, and analyzed an aliquot of each extract by LC-UV. The method exhibited poor recovery but acceptable between-laboratory precision for

metribuzin DADK and metribuzin DK (Table 2.15). The method-performances for carbofuran phenol and linuron were calculated to be significantly different for reagent water and finished drinking water. In reagent water, the overall CV range was 7.8–24.1% for all 18 compounds, and 5.5–38.6% in finished drinking water. In conclusion, this method was recommended as a first action for AOAC. It was reported that improvements are needed in the optimization of resolution between closely eluting peaks, since a single column could not completely separate the 18 analytes. Also, improvements in the recoveries for certain analytes are needed in the future.

A collaborative study has been undertaken for the determination of ethylene thiourea (ETU) in finished drinking waters, using the GC-nitrogen-phosphorus detector (NPD), according to US EPA National Pesticide Survey method 6 [94]. As in the previous studies reported [92,93], the CV varied from 5 to 24% in reagent water. The studies for the two water matrices showed no statistically significant results, and the method has also been adopted as a first action by AOAC International for the determination of ETU in finished drinking waters.

Finally, the EPA undertakes various interlaboratory exercises using LC-MS tech-

TABLE 2.15

ACCEPTANCE LIMITS FOR THE ANALYTES OF A LABORATORY QUALITY CONTROL SAMPLE, AS PERCENT OF SPIKED VALUE % (EPA NATIONAL PESTICIDE SURVEY METHOD 4 OF PESTICIDES IN REAGENT WATER)

Analyte	Spike value ($\mu\text{g/l}$)	Recovery (%)	Overall SD	Acceptance limits
Atrazine dealkylated	5	4.48	0.82	40–139
Barban	10	8.84	1.31	49–128
Carbofuran phenol	50	37.5	8.00	27–123
Cyanazine	10	9.16	1.74	39–144
Diuron	1.0	1.02	0.13	63–141
Fenamiphos sulfone	100	96.4	11.7	61–132
Fenamiphos sulfoxide	20.0	19.5	2.19	65–130
Fluometuron	2.0	1.76	0.29	45–132
3-Ketocarbofuran phenol	5.0	4.38	0.83	38–137
Linuron	2	1.89	0.14	74–116
Metribuzin DA	5.0	4.06	0.66	42–121
Metribuzin DADK	5.0	1.17	0.20	11–35
Metribuzin DK	5.0	1.95	0.34	19–59
Neburon	2.0	1.81	0.20	61–121
Pronamide metabolite	10.0	10.3	1.49	58–148
Propanil	1.0	0.98	0.11	65–131
Propham	10.0	8.63	2.08	24–149
Swep	10.0	9.51	0.81	71–119

The acceptance limits are %, defined as (mean recovery \pm 3 standard deviations)/spike level. The spike level is 15 times the method detection limit (MDL).

TABLE 2.16
ACCURACY AS MEAN PERCENT RECOVERY (PRECISION AS CV OF CHLORINATED
HERBICIDE INTERLABORATORY DATA AT THREE DIFFERENT CONCENTRATION LEVELS)

Compounds	LC/PB-MS			LC/TSP-MS ($\mu\text{g/ml}$)		
	500	50	5	500	50	5
2,4,5-T	109 (14)	63 (33)	223 (2)	90 (23)	62 (68)	90 (28)
2,4-D	111 (14)	85 (36)	270 (30)	86 (17)	64 (80)	103 (31)
2,4-DB	120 (13)	72 (30)	207 (14)	195 (22)	104 (28)	96 (21)
Dalapon	ND	ND	ND	83 (13)	121(99)	150 (4)
Dicamba	95 (24)	73 (89)	ND	77 (25)	90 (23)	105 (12)
Dichlorprop	111 (13)	101 (24)	323 (19)	84 (20)	96 (15)	102 (22)
Dinoseb	63 (13)	30 (3)	ND	78 (15)	86 (57)	108 (30)
MCPA	111 (20)	106 (25)	280 (10)	89 (11)	96 (20)	94 (18)
MCPP	107 (17)	101 (37)	290 (8)	86 (12)	76 (74)	98 (15)
Silvex	122 (20)	72 (45)	220 (6)	96 (27)	65 (71)	87 (15)

ND, not detected

niques for the determination of chlorinated phenoxy acids and carbamates using test solutions [94,97]. These were remarkable in that they were the first interlaboratory studies using LC-MS interfacing systems and showed the variability of results using different interfaces (see Table 2.16). With thermospray LC-MS the interlaboratory method precision ranged from 6.5 to 33.1% whereas the interlaboratory method precision ranged from 29.8 to 98.2% relative standard deviation, indicating the much higher deviation when using LC-MS compared to other detectors such as UV. There are many operational parameters that could contribute to the high interlaboratory variability, but especially the thermospray tip temperature which plays a major role in the fragmentation of thermally labile carbamates and needs to be carefully controlled.

The quantitation problems using different LC-MS approaches have been reported recently in interlaboratory comparison studies between thermospray and particle beam LC-MS. The difficulties encountered with current LC-MS instrumentation in validating the results of three interlaboratory EPA studies, including a LC/TSP-MS study for chlorinated herbicides, a LC/TSP-MS study for carbamate pesticides, and a LC/PB-MS study for carbaryl, linuron and benazidines, among other compounds, were reported in a summary paper [97] and previous papers from the same authors [95,96]. The LC-MS interlaboratory studies performed so far by the US EPA have utilized TSP- and PB-LC/MS interfaces.

One of the difficulties encountered in conducting interlaboratory studies on emerging technologies is constraining the participants, who frequently use instruments from a variety of manufacturers, to a rigid set of conditions. The state of technology in the field of LC-MS is such that different instruments behave differently under supposedly "similar" conditions. The complexity of the instrumentation leads

to many variables which contribute to performance. Therefore, the EPA has established working groups to identify which single-laboratory-evaluated methods should be evaluated in interlaboratory studies. One important issue is selecting the participants for the study and ensuring that they follow the Association of Official Analytical Chemists' guidelines. In these interlaboratory studies, various types of TSP interfaces were used and two types of PB interfaces (pneumatic and heated pneumatic) were also used by participants. There were eight simulated sample extracts and one blank extract sent to the participants. The simulated sample extracts consisted of duplicate extracts containing the 11 chlorinated herbicides at four concentration levels (5, 50, 250, and 500 $\mu\text{g/ml}$). The participants of Study 2 received nine simulated sample extracts, containing the nine carbamates at four concentration levels (5, 35, 40 and 90 $\mu\text{g/ml}$), and one blank extract.

The interlaboratory evaluation showed that differences in each of the manufacturers' TSP made it impractical, and sometimes impossible, to impose strict guidelines for the operating conditions of the TSP interface (e.g., tip temperature, repeller voltage, discharge electrode, filament on/off). Instead, for the TSP studies, the participants were asked to follow the manufacturer's recommendations for optimal interface performance. Some guidance was given to the participants as to approximately what tip temperatures to use, whether the filament-on mode of operation would help sensitivity, whether a repeller voltage was necessary, and what ionization mode to use (negative versus positive).

In another interlaboratory study, the LC conditions (e.g., flow rate, gradient programming, buffer strength) were specified, but few laboratories were able (or willing) to follow the guidelines. Because of the variations used in the gradient programming and, more importantly, the differing buffer strengths, large differences in chromatography could be seen. Four laboratories out of nine followed the chromatography programming exactly; these laboratories' chromatograms were similar to those obtained from the single-laboratory evaluation. One laboratory in particular used a different buffer strength; this caused a very marked difference in analyte separations and retention times. Because of these data, the LC conditions for the carbamates were rewritten and incorporated into Method 8321. Another source of variation was the TSP tip temperature. Usually, the optimal temperature is approximately 5–10°C cooler than the temperature of complete vaporization; for a given interface (regardless of manufacturer), this temperature is usually instrument-specific. A comparison of the overall accuracy (mean percent recovery) and precision (percent relative standard deviation) between PB and TSP is shown in Table 2.16. At high concentration levels (500 $\mu\text{g/ml}$), PB has a tendency to give biased high results, while TSP tends to give biased low results. At the very low concentration level (5 $\mu\text{g/ml}$), only one PB laboratory could detect the analytes, but the reported values were twice as high as the "true" value. Two of the three laboratories using the TSP interface reported results at the 5 $\mu\text{g/ml}$ level. The results showed a very low CV (+3%) and average CV of 19%. In general, it can be said that PB gives better precision than

TSP, particularly at the high concentration level (500 $\mu\text{g/ml}$), while TSP is the more sensitive technique (for the chlorinated herbicides).

As a general conclusion, from the interlaboratory studies conducted by the US EPA, we can say that the overall relative standard deviation is generally higher in finished drinking water than in reagent water, which indicates a dependence of the matrix. This overall CV can reach values up to 34–38%, either in GC or HPLC, which is above the 25% we reported in the previous examples and exceeds the 22% recommended by AOAC [76]. This indicates, once more, that the value of 17% imposed by the Aquacheck interlaboratory program is clearly restricted. Certainly, and following the EPA results, the Aquacheck single-flagged results of 34% would be appropriate for conducting interlaboratory studies of pesticides in various water matrices. As regards interlaboratory studies using LC-MS, the overall CV can approach 100%, which clearly indicates that most of the previous rules and recommendations were changed because of instrumental difficulties. Only using the recently developed APCI techniques, the results achieved in a recent interlaboratory exercise were promising [89]. This is probably related to the much greater sensitivity of APCI compared to previous interfacing systems such as thermospray and PB. Because of the current use and application of LC-MS interfacing systems in the analysis of polar pesticides, it is recommended that many more interlaboratory studies using the different types of interfaces should be undertaken in the near future, always starting from standard solutions and then analyzing spiked water samples.

2.3.7. Other interlaboratory programmes

In this section, we discuss other interlaboratory exercises on polar pesticides, organized in Europe, in which the authors of this book have participated. One of us (D.B.) has been involved for several years in MED POL pilot surveys of various classes of compounds (organophosphorus pesticides, herbicides and fungicides) to be monitored in coastal waters of the Mediterranean region. In the monitoring programme, method performance studies were undertaken. All the laboratories from the Mediterranean countries participating in the pilot survey applied an identical analytical protocol, so the results achieved in the monitoring survey will be easier to intercompare [98–100]. Figure 2.9 shows the results obtained in the fungicide interlaboratory exercises with five participating laboratories. This cannot be considered a “true” interlaboratory exercise, since the number of laboratories is too low, but the problem is that in Mediterranean countries other than Spain, Italy, France, Greece and Monaco, it was difficult to find laboratories that could do this job. Analytical protocols for analyzing the fungicide solutions were adopted according to Ref. [99]. The protocol for the interlaboratory exercise was similar to that of Aquacheck; ampoules containing solutions of fungicide standards in ethyl acetate (to avoid degradation), with full instructions for the analysis, and data reporting forms, were distributed to the participants. The analysts were instructed to remove 0.2 ml aliquot solu-

tions from the two ampoules, mix each with 1 ml of acetone (to facilitate dissolution) and add 1.0 l of doubly distilled water. The reconstituted solutions were then to be analyzed by the accepted protocol [99]. One of the solutions was analyzed by GC-ECD and another one by GC-NPD. Triplicate analyses were requested. From the results shown in Fig. 2.9 it can be seen that good agreement is generally found between laboratories. For folpet, however, two laboratories reported substantially elevated concentrations. The most likely explanation for this would be that either there is a co-eluting peak in the GC-ECD chromatogram, or that imprecision was introduced owing to the small size of the folpet peak. It is also noteworthy that two laboratories report low concentrations of chlorothalonil. It was noticed that this component in standard mixtures (at low concentrations) is susceptible to degradation, which would appear a likely explanation for these values. For captafol and captan, the CV was below 25%, whereas in the other cases the CV are of the order of 40%, except for folpet which were higher.

The other example reported here was discussed briefly in a recent book chapter [87] but one of our laboratories has participated in this exercise, so the data are reported in Table 2.17. This interlaboratory exercise was sponsored by Ciba, and five water samples were submitted to 11 laboratories for determination of atrazine. The samples were prepared in Switzerland and were taken from a lysimeter. The soil history indicated that the soil had not been in contact with atrazine for at least 3 years, and measurement concentrations of atrazine in the leachate indicated that atrazine was below the limit of detection. A 15 l sample of leachate was transferred to 20 l container equipped with an agitator. Then 1.5 ml of the 1 $\mu\text{g/l}$ atrazine stock solution was added and mixed during 30 min. Portions (1 l) of this solution were put into 1 l polycarbonate flasks and immediately deep-frozen. The same procedure was repeated for other triazine derivatives. All the participating laboratories were asked to measure the atrazine content of the five ground water samples using their own atrazine method. From the results shown in Table 2.17, it can be deduced that only three laboratories were able to find atrazine in only one sample. Recoveries varied between 85 and 110% of the amount added (0.1 $\mu\text{g/l}$) and the results for the other four samples were below their limit of determination. Three laboratories detected practically no atrazine in all five samples and five laboratories measured relatively high concentrations of atrazine in all samples. The interlaboratory comparison method showed that, with the common atrazine methods used by different laboratories, a correct determination of atrazine at the low ppb level in water samples is quite difficult. The results of this intercomparison were taken by Ciba to demonstrate that most of the atrazine levels reported in Europe were incorrect. However, we should point out that, taking into consideration the participants in this interlaboratory exercise, many known laboratories performing monitoring of atrazine in drinking water were not involved. So the statement given by Ciba is not valid, since it represents only some of the laboratories in Europe but not the laboratories most currently involved in the analysis of atrazine in water.

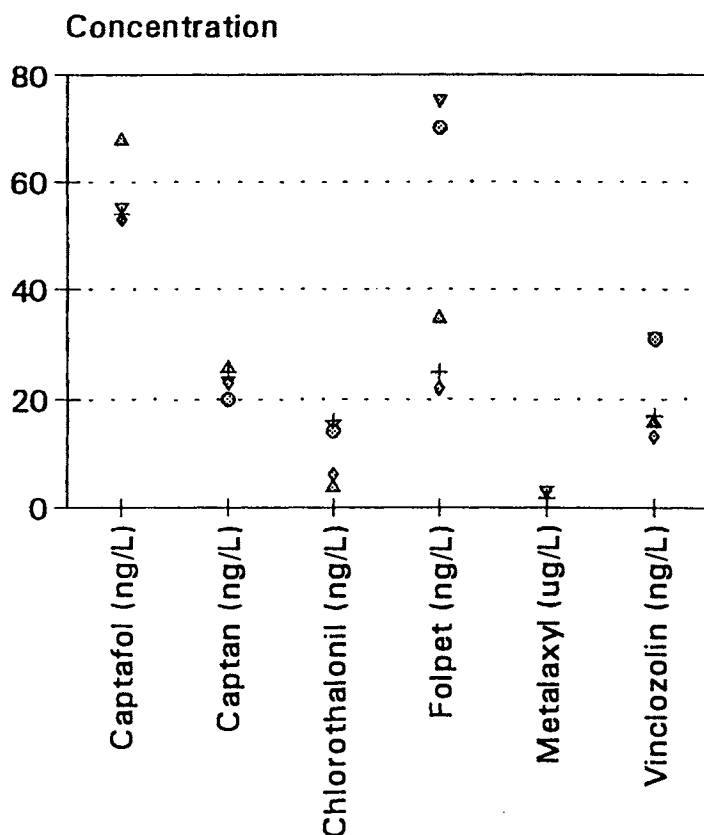


Fig. 2.9. Fungicide intercomparison organized by the International Atomic Energy Agency.

2.3.8. Conclusions

From the examples we have discussed, it is clear that interlaboratory studies of polar pesticides are still needed. These studies can be undertaken in different ways, as method performance studies, which fix the method of analysis in advance, or as performance studies in which each laboratory uses its own method. Certainly, interlaboratory studies for pesticides should start analyzing standard pesticide solutions and comparing the most commonly used methods, GC and HPLC. The use of immunoassay methods is also envisaged, and should be undertaken in order to validate the increasing application of biological techniques. In a second step, polar pesticides spiked in water samples using different approaches, either using freeze-drying or a standard solution which is spiked afterwards are proved. Future studies will also be conducted in which the pesticides are preserved in SPE materials, so the transport of samples to laboratories will be easier. There is no agreement about the overall CV of

TABLE 2.17
RESULTS MEASURED FOR ATRAZINE CONTENT, IN $\mu\text{g/l}$

Laboratory	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
A	0.08	<0.01	<0.05	<0.05	<0.05
B	0.281	0.613	0.149	0.244	0.225
C	0.16	0.24	0.29	0.13	0.088
D	<0.05	<0.05	0.085	<0.05	<0.05
E	0.04	<0.04	0.11	0.21	<0.04
F	<0.05	<0.05	0.11	<0.05	<0.05
G	<0.005	<0.005	0.11	<0.005	<0.005
H	0.64	0.80	0.86	0.54	0.67
I	<0.02	<0.02	0.06	0.02	0.02
K	4.64	4.51	0.82	4.41	0.96
L	<0.05	<0.05	<0.05	<0.05	<0.05

Only sample 3 contained atrazine, at the $0.1 \mu\text{g/l}$ level.

Laboratories A, F and K are Spanish; laboratories B and G are Danish; laboratories C, D and H are German; laboratory E is Dutch; laboratory I is French and laboratory L is Swiss.

the interlaboratory studies for polar pesticides. Whereas the Aquachek organization has imposed very strict limits of 5% and 17% for pesticide standards and spiked water samples, respectively, the European Commission, through the BCR, has more flexible limits reflecting the state of the art. In this respect, acceptance values for standard solutions are in the range of 12.5%, whereas for spiked samples the overall precision is in the range of 25%. The US EPA distinguishes between different spiked waters, and indicates that as the water matrix becomes more complex, the overall standard deviation is also increased. Although for reagent water samples spiked with pesticides the overall CV is in the range of 25%, when finished drinking water is considered, the precision of the method reaches values close to 40%. The method showing less precision than reported in the above-mentioned examples was in the determination of standard solutions of pesticides by LC-MS. In that case the precision went from 29 to 98%, indicating the instrumental difficulties encountered with this technique, which requires various steps of optimization. The use of novel interfacing systems such as APCI seems likely to reduce the precision, but still comparative studies using various LC-MS instruments with APCI techniques have not been undertaken. This chapter shows that interlaboratory studies of pesticides are still needed, and this is probably one of the areas that will grow in the next few years.

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Chromatographic and Related Techniques for the Analysis and Detection of Pesticides

3.1. INTRODUCTION

Chromatographic techniques are the common methods of choice for the determination of pesticide residues and their metabolites in environmental waters. Pesticides of a wide range of polarities often need to be measured at extremely low concentrations in complex environmental matrices. The general scheme for the trace-determination of pesticides in water involves the sampling, sample pretreatment, and proper analysis followed by identification, confirmation and quantification. The reader may wonder why we have decided to examine the separation and identification steps before the pretreatment. There are several reasons for this approach. First, the three steps, pretreatment, separation and detection/quantification are closely related and should always be considered as a whole, even when they are not coupled on-line. For example, when a very sensitive and selective detection method exists, e.g., a post-column reaction followed by fluorescence detection as is used for *N*-methylcarbamates, the sample pretreatment can be simplified and a small sample volume will be required for trace enrichment. In contrast, for the determination of thermally labile compounds in complex matrices which can only be analyzed by LC-UV, then a high sample volume and a clean-up step will be required. Therefore, the sample pretreatment depends strongly on the separation and detection steps. The compatibility of the steps should be also considered. For example, the choice of solvent used to dissolve the sample extract will depend on whether the analysis is performed by GC or LC. Secondly, looking first at the separation step corresponds to the way in which chemists work in the laboratory. Once the separation and detection are performed, the detection limit is measured for the compounds of interest, thus allowing one to estimate the sample volume needed for enrichment taking matrix effects into account. The sample volume required will provide a guide for selecting the best available sample pre-treatment technique.

Separations of pesticides by chromatographic methods is now routine prior to de-

termining individual components. Gas chromatography (GC) and liquid chromatography (LC) are the methods of choice to separate complex mixtures prior to detection. Thin-layer chromatography (TLC), which was popular in the late 1960s and 1970s, has been almost completely superseded by LC and GC. However, thanks to recent improvements in instrumentation and especially in detection, TLC is being used more than in the recent past, and is the object of an official method. Capillary zone electrophoresis (CZE) is certainly the emerging technique of recent years with relevant new applications in environmental analysis. It has been shown to be complementary to LC and GC for some pesticides, and of real value. Although it is claimed to be a promising technique, supercritical fluid chromatography has not really found its place in environmental analytical laboratories, partly because of its higher demands and because many published applications can be performed using GC or LC. In contrast, the use of supercritical fluids for extraction of pesticides in various matrices has already been approved in some EPA methods, and is thoroughly discussed in the next chapter.

GC has for a long time been the method of choice for the determination of the first generation of pesticides, i.e., organochlorine and organophosphorus pesticides. Their strong hydrophobic character, their sufficient volatility, and their high thermal stability make them easily amenable to GC analysis using the highly sensitive electron capture detector (ECD), nitrogen-phosphorus detector (NPD), or mass spectrometer (MS), among others. Up to the early 1970s, the trace determination of pesticides was mainly devoted to these classes. In the late 1970s problems were raised regarding the analysis of thermally labile and/or polar pesticides, and LC was the technique that received some attention [1]. Large improvements have been made in LC detection techniques in the past decade, with a reduction of detection limits of UV, diode array (DAD), fluorescence, and MS detectors so that now LC and GC are both used in trace analysis. In some cases, the question of whether to use GC or LC is answered very clearly, because some compounds such as lindane cannot be analyzed by LC and other thermally unstable compounds can only be analyzed by LC techniques. For other compounds, the choice is not so clear and the two methods may appear to be in competition. The use of GC will require the use of derivatization techniques and further analysis with a selective detector, generally with good detection limits, and it will be selected by some chemists, whereas others will prefer LC without prior derivatization, even if the detection limits are a little higher. For the same reason, i.e., the fact that the analytes can be analyzed without prior derivatization, multiresidue analyses will be performed better using LC. However, in most cases, LC and GC are fully complementary techniques, and both must be available in any environmental laboratory performing pesticide analyses. The two methods were compared recently in a review, for the analysis of four selected groups of pesticides including the polar compounds, carbamates, triazines, phenylureas, and phenoxy-acetic acid derivatives [2]. We should point out that, in practice, the method of choice from GC or LC will depend strongly on the experience and preferences of the chemist and the traditions

of the laboratory. Probably this is the best solution, since in this way more accurate determinations of a target analyte can be performed.

In this chapter, we do not describe in detail the basis of the techniques of separation; the literature contains many general reviews and books. Emphasis is placed on how complementary the methods are and on the selectivity that can be provided by the detection mode, either alone or by some combination of the separation and detection mode. Not all the methods that have been described are given, but only a selection of the techniques which, in the authors' experience are robust and reliable. Multiresidue possibilities are examined particularly. In order to avoid false positive determinations, it is compulsory to use confirmation methods. In this sense, the use of MS techniques and/or the use of two different GC or LC columns of different polarities for confirmation of pesticides are emphasized. Modern TLC and CZE are discussed along with specific applications.

Mass spectrometry (MS) has now become a powerful tool for identification and quantification, and is increasingly found in environmental laboratories, coupled to GC, LC and also to CZE. Bench-top MS instruments for use with GC and LC are commercially available and simplify the unequivocal identification of pesticides in environmental matrices. The fact that prices have gone down during the last few years has helped to increase the numbers of GC-MS and LC-MS systems allocated in many routine laboratories. The use of CZE-MS is still being developed, but some specific applications to quaternary ammonium pesticides and to highly ionic molecules can easily be performed. This coupling technique will also grow in the near future. The use of MS techniques coupled to chromatographic methods is discussed in the second half of this chapter, since they need specific attention.

3.2. GAS CHROMATOGRAPHY

Gas chromatography (GC) is by far the most frequently used chromatographic method in the analysis of pesticides in water. This can easily be seen from the descriptions of the procedures used in official methods such as the EPA or NPS methods [2–6]. The high resolving power of capillary GC columns, the high sensitivity and sensibility of the GC detectors, and the ability to connect a MS allows residue-determinations for a wide range of pesticides at the $\mu\text{g/l}$ levels. However, the sensitivity is not high enough to achieve the limits of quantification required by the Drinking Water Directive of the Commission of the European Communities (DWD-CEC) for individual pesticides, so a preconcentration step is always necessary.

GC can be applied to many pesticide classes, but a number of classes are not amenable to GC because they have too low volatility or show thermal instability. As examples, most of the acidic herbicides, many polar herbicides such as paraquat, diquat and glyphosate, some phenylurea and sulfonylurea herbicides, most of the carbamate insecticides and herbicides, and benzimidazole fungicides cannot be analyzed directly by GC. Some of these can be determined by GC, provided a derivati-

zation step, such as esterification or alkylation, is performed prior to chromatography.

Modern instruments permit the use of various injection and detection modes. For a long time, the conventional injected volume could not exceed 1 μl . Now, thanks to the use of the retention gap, on-column injection, and special interfaces, this volume can be increased. Solid-phase extraction (SPE) can be coupled to GC, allowing the injection of several hundreds of microlitres, via an SPE-GC system, discussed in Chapter 5, which deals with fully on-line techniques.

3.2.1. Selection of columns, injectors and detectors

3.2.1.1. Selection of the stationary phase and column

The most common columns are fused silica open tubular (FSOT) capillary columns with internal diameters varying in the 0.2–0.7 mm range and with lengths of 10–50 m. They became available in the early 1980s. The technology used to prepare the fused silica surface, to coat it with the phase, and carry to out the cross-linking and chemical bonding of the stationary phase, in situ, to the residual hydroxyl groups on the silica column wall, has reached a high degree of perfection. Commercial columns are now very robust in terms of efficiency, stability, low bleed, inertness, and uniformity.

A wide range of stationary phases is available. The selection of the stationary phase is guided by the rule “like-to-like”, meaning that for apolar analytes a non-polar phase will be selected. The most apolar phase is obtained with the cross-linking and chemical bonding of a polymer phase based on linear methyl silicones. An increase in the polarity of the stationary phase is obtained by substitution of a proportion of the groups with more polar moieties such as phenyl, trifluoropropyl or phenylcyanopropyl. Open capillary columns are classified by the McReynolds polarity scale, which was first introduced to classify the earlier packed-column phases on the basis of the relative retentions of five probe analytes. However, when the compounds to be separated have a wide range of polarity, the column selection is more difficult and more empirical. Commercial catalogues contain many examples of pesticide separations, especially those corresponding to the EPA methods. Table 3.1 gives the polarity equivalent of the main commercially available columns, with some selected applications or references to EPA methods.

When possible, it is better to select low polarity columns, which are generally more stable than the more polar ones. In fact, they are the primary choice for screening pesticides. In order to avoid false positives, it is necessary to use confirmation methods. In this sense, the use of MS is preferred, but, in its absence, one should use two different columns of different polarity, the primary column and a confirmatory column. A more polar column is generally used for confirmatory purposes. Limiting factors can be the column's stability and bleed at high temperature. This can occur for pesticides such as the synthetic pyrethroids, which are only eluted at high temperatures.

TABLE 3.1
CHARACTERISTICS OF THE BONDED PHASES USED AS CAPILLARY GC COLUMNS

Stationary phases	Commercial equivalent ^a	Selected applications in pesticide analysis
100% Dimethylpolysiloxane <i>Non-polar</i>	DB-1, HP-1, SPB-1, CP-Sil 5CB, Rtx-1RSL-150, BP-1, CB-1, OV-1, PE-1, SE-30, Ultra-1	EPA 505 for organohalide pesticides EPA 1618 for organophosphorus insecticides EPA 551 for halogenated hydrocarbons
(5% Phenyl)-methylpolysiloxane <i>Low-polarity</i>	DB-5, HP-5, HP-101, SPB-5, CP-Sil 8CB, Rtx-5, RSL-200, BP-5, CB-5, OV-5, PE-5, SE-52, Ultra-2, DB-5ms, HP-5MS, PTE-5	EPA 508 for organochlorine pesticides EPA 525 for organic compounds in water EPA 515 for chlorinated acids EPA 548 for endosulfan pentachlorophenol Pyrethroid insecticides
(50%-Phenyl)-methylpolysiloxane <i>Midpolarity</i>	DB-17, HP-17, SPB-50, CP-Sil 8CB, Rtx-50, RSL-300, SP-2250	Triazine herbicides EPA 505 for organohalide pesticides and aroclors
(35%-Phenyl)-methylpolysiloxane <i>Midpolarity</i>	DB-35, Rtx-35, SPB-35, AT-35, Sup-Herb	EPA 507 for nitrogen containing herbicides Organochlorine pesticides Triazine herbicides
(6%-Cyanopropyl-phenyl)-methylpolysiloxane <i>Midpolarity</i>	DB-1301, Rtx-1301, CP-624, PE-1301, HP-1301	Mid-polarity herbicides
(14%-Cyanopropyl-phenyl)-methylpolysiloxane <i>Midpolarity</i>	DB-1701, SPB-1701, CP-Sil 19CB, Rtx-1701, OV-1701, PE-1701	Organophosphorus insecticides EPA 508 for organochlorine pesticides Phenoxyacid herbicides Triazine herbicides EPA 552 for haloacetic acid and dalapon
Polyethylene glycol MW = 20000 <i>High polarity</i>	DB-WAX, Carbowax, Stabilwax, HP-20M, CP-WAX 52CB, CB-WAX, BP-20, SUPELCOWAX 10	NPS Method 6 for ethylenethiourea Amines Alcohols Aldehydes

^aEquivalents taken from commercial reference catalogues.

Different inner diameters are available for the columns, from 0.7 to 0.5 mm “megabore”, for increased sample capacity, to 0.05 mm “microbore” for ultra-high speed with high resolution. The resolution depends on the square root of the column length and, at a first approximation, on the inverse of the column diameter. There-

fore, for some separations, the use of a smaller inner diameter can be more effective than increasing the column length. The thickness of stationary phase film has an effect on the retention and the sample-capacity. Very thin films should be avoided for labile pesticides; thick films provide a higher degree of deactivation of the column wall, but they have the disadvantage of bleed, and provide steeper van Deemter curves. A good average selection for screening is a 25–30 m column with a 0.2–0.3 mm i.d. and a 0.15–0.33 μm film.

3.2.1.2. *Sample introduction systems*

In trace analysis, after the off-line extraction, only an aliquot of the extract is injected onto the GC. It is important to introduce the largest possible amount, so that the enrichment factor is higher. As a general rule, when one is comparing methods, one should consider not only the absolute amounts detected by the system, also called the instrumental detection limits (IDL), but also the total amount that can be injected by the system. In this respect, the so-called method detection limit (MDL), which also includes the extraction step, is preferred over the IDL, since it gives a better estimation of the possibilities of an analytical technique. A second general comment is that the sample introduction is not always straightforward and should be considered carefully in the GC system, owing to its importance for quantitative measurements. It should not generate discrimination or degradation of samples, nor introduce any band broadening. Three techniques are mainly used for trace analysis: the cold on-column, the hot-splitless, and programmed-temperature-vaporizer injectors. The direct coupling with trace enrichment requires a special interface and is discussed in Chapter 5.

One important effect of injecting high sample volumes onto a capillary column is to achieve a focusing of the sample analytes into a narrow band at the head of the column prior to their volatilization for separation. This point has been extensively discussed by Grob [7,8].

The splitless injector is used in one common technique for pesticide residue analysis. Rapid vaporization of the injected sample occurs in a high temperature injector with the split valve closed, and with the column temperature below the boiling point of the solvent, in order to focus the analyte. Then the split valve is opened, to flush the solvent from the injector, and the temperature of the column is increased. Optimization of the operating conditions has been shown to depend strongly on the injector insert's design. Solvents having higher boiling points, such as iso-octane, cyclohexane or ethyl acetate, should be preferred in order to ensure a better separation of the solvent from the early-eluting compounds. It is also recommended that the standards and sample should be in the same solvent, and the injected volumes identical. The usual sample volumes are 1–2 μl . This type of injector has the advantage of preventing the non-volatile compounds from entering the columns, but suffers from the drawbacks of generating degradation and discrimination problems. The discrimination can be reduced by using fast automated injection.

The cool on-column injector allows the injection of analytes with minimum degradation. The main advantage for pesticides is the reduced thermal decomposition. Some thermally degradable pesticides, such as isoproturon and other phenylureas, became amenable to GC thanks to this injector. The sample is introduced directly into the head of the column which is raised to a temperature slightly below the boiling point of the solvent. For "large" sample volumes ($>1\ \mu\text{l}$), the injection should be performed slowly; the solvent is deposited in a flooded zone at the head of the column, and the sample components are focused at the tail of the film of solvent which evaporates. Then, raising the column temperature allows the vaporization of the compounds. However, several injections of real sample extracts will rapidly degrade the chromatographic performance of the column. A retention gap consisting of 1–10 m of uncoated column is required for a better solvent effect with injection volumes above $2\ \mu\text{l}$. The sample volume could be increased to 50–200 μl by using longer retention gaps. When the chromatographic performance deteriorates, it can be restored by simply rinsing the retention gap or by removing the first few centimetres.

The programmed temperature vaporization injector is similar to a splitless injector, but with injection performed at a low temperature. It can be heated rapidly and cooled independently from the column, using a separate programme for heating. The injection can be made with a solvent venting, allowing injection of larger volumes, up to 200–250 μl . After cold injection, the start temperature is below the boiling temperature of the solvent and the solvent is vented through the open split-valve. After closing the valve, the sample is evaporated by very rapid heating of the injector. A main disadvantage is the possible loss of the more volatile analytes during the solvent-venting.

Even if the sample volume can be increased, as seen above, in many instances it is of the order of μl . Since reproducibility in the injected volume is difficult to achieve, any method should include an internal standard which is added to the extract and allows quantitative measurements to be made independently of any knowledge of the exact volume injected.

3.2.1.3. *Common GC detectors for pesticides (except mass spectrometry)*

The GC detectors are often classified on the basis of their selectivity. Universal detectors respond to every component, and selective detectors to a group of compounds with similar characteristics.

The most important characteristics of a detector are the response factor, sensitivity, minimum detectability, linearity of response, selectivity, and quantitative information. The response factor is usually expressed as an amount of signal per unit of mass sample and it allows one to compare the responses of various analytes. The detector responses can be dependent on the sample mass flow, as with flame-based detectors which consume the sample, or on sample concentration, as is the case with electron capture detectors. However, since a make-up gas is usually added, a rather uniform sample concentration is observed in the gas for most of the detectors and the

detector responses can be compared on a mass flow basis. Regarding the minimum detectability, one has to distinguish between the limit of detection (LOD) and the limit of quantification (LOQ). The LOD is defined as a detection signal equal to two or three times the noise level (signal-to-noise ratio). The LOQ is determined with real samples being put through the complete procedure, including the sample pre-treatment, and with a signal-to-noise ratio of 5–10 times. The LOD and LOQ depend on the sample matrix and on the efficiency of the sample pre-treatment to remove any matrix effect. For routine analysis, it is commonly admitted that for a quantification at the $0.1 \mu\text{g/l}$ level according to the EEC regulations, the LOD should be as low as $0.01\text{--}0.02 \mu\text{g/l}$. The linear range is important for quantification, indicating the range where the response factor is constant. The selectivity of a detector towards given compounds is the ratio of their respective response factors. When the response factors are similar for a wide range of compounds, the detector is non-selective. This is the case for the flame ionization detector (FID).

Table 3.2 gathers the GC detector specifications. For trace level detection of pesticides, selective detectors offer the most effective approach and are usually preferred over the FID, although the advantages of the latter are numerous, i.e., it is easy-to-use and has a fast response, good stability, wide linear range, good sensitivity and a universal response to organic compounds.

The electron-capture detector (ECD) was the first selective detector to be used extensively for organochlorine pesticides. Modern ECDs use ^{63}Ni sources which can be used at temperatures up to 400°C , are stable, and can provide a linear range over a factor of 10^3 . The principle of this detector is based on the reduction in conductivity caused when the electrons are captured by specific analytes. The ECD is extremely sensitive for polychlorinated (and other polyhalogenated) pesticides, with LOD in the sub-picogram range. The sensitivity is lower for low halogenation levels, and it is not suitable for monochloro compounds. This detector is appropriate for drinking water and ground water, but when more complex matrices, such as surface water extracts, are injected the selectivity is reduced since many organic compounds can interfere with the ECDs response, to a greater or lesser extent. An extensive sample preparation of the samples may then be necessary.

The nitrogen-phosphorus detector (NPD), also named an alkali-thermoionic, or alkali-flame ionization detector, uses a hydrogen plasma acting on an alkali salt source, so that nitrogen- and phosphorus-containing compounds are selectively ionized. This detector is used widely in pesticide analysis, owing to its high sensitivity and its high selectivity against carbon-, hydrogen-, and oxygen-containing compounds. The reason is that many pesticides contain nitrogen or phosphorus atoms in their structures (organophosphorus, triazines, etc.). The NPD is not as stable as FID and requires frequent calibration. There are different configurations of NPD detectors, according to the different manufacturers, and they can exhibit a flame or not.

The flame-photometric detector (FPD) is selective towards phosphorus- and sul-

TABLE 3.2
SPECIFICATIONS OF THE GC DETECTORS USED IN PESTICIDE ANALYSIS

Detector	Element of functional group	Sensitivity ^a (pg/s)	Linearity
Flame ionization (FID)	Universal (C–H)	10	10 ⁷
Electron capture (ECD)	Compounds capturing electrons, i.e., polyhalogens	0.1 (lindane)	10 ³
Flame photometric (FPD)	P	0.5–0.9	5 × 10 ⁴
	S	5–20	5 × 10 ²
Nitrogen-phosphorus (NPD)	N	0.2–0.4	10 ⁵
	P	0.1–0.2	10 ⁴
Atomic emission (AED)	C	0.5	10 ⁴
	H	2.2	
	S	1.7	
	P	1.5	
	F	40	
	Cl	39	
	Br	79	
	O	75	
	N	7	
	Hg	0.1	
	Sn	1	
Photoionization (PID)	Aromatics	100	
Electrolytic conductivity (ELCD)	Cl, Br	0.2–1	10 ⁶
	N	2–5	10 ⁴
	S	2–5	

Selected values from Refs. [9–11].

^aFor AED, from Ref. [9].

fur-containing compounds, and is more sensitive for the phosphorus-containing compounds.

Electrolytic-conductivity detectors (ELCD), although very selective for halogenated compounds, and photoionization detectors (PID) which are selective for aromatic compounds, are rarely used in pesticide analysis.

Recent publications have pointed out the advantages of the atomic emission detector (AED). The detectors using a microwave-induced helium plasma with an atomic emission spectrophotometer are element-selective [9]. Therefore, this technique allows one to detect any element selectively. Recent AEDs include photodiode array-based spectrophotometers which permit the simultaneous multi-wavelength detection. The sensitivity depends on the element, as shown in Table 3.2. The differences from other existing element-specific detectors come from the importance of inter-element and inter-functional group selectivity. For example, the NPD is sensitive for both N and P but cannot distinguish between these elements, whereas the AED can monitor only P without interference from other elements. The high selectivity of element-specific detection allows a reliable identification of pesti-

cide containing indicative hetero-elements. Interesting applications have been obtained for S-containing pesticides, mainly some organophosphorus compounds, as well as for those containing the elements Hg or Sn. In general, heteroatoms such as fluorine, chlorine, bromine, nitrogen, sulfur and phosphorus which are important in pesticide residues are of interest. However, one drawback of the AED is its relatively low sensitivity, especially for nitrogen. For this reason, some applications require the injection of larger volumes of the sample extracts using the PVT detector, or a higher concentration of sample extracts, because matrix compounds are usually observed in the carbon, hydrogen and oxygen traces [12]. Another option is to use an on-line preconcentration system, as developed by the Brinkman group. An advantage of the AED detector is the possibility of multiresidue analysis.

Hyphenated systems which use techniques to allow the selective recognition of the separated compounds such as GC-mass spectrometry, GC-Fourier transform infrared spectroscopy (GC-FTIR) and GC-AED, are the best analytical techniques available for the identification of unknowns which produce complementary information. GC-FTIR often complements MS data by providing additional information for isomer and structure identification. Since FTIR is a non-destructive method, the on-line combination GC-FTIR-MS has recently become commercially available and is certainly the most powerful tool for identification.

3.2.2. General considerations about analytical procedures

The most complete guidelines for describing whole GC procedures for pesticide analysis are given in the EPA methods. They include: (i) the acceptance of recoveries for the extraction method of 70 up to 130%, with a maximum relative standard deviation of 30% each; (ii) some recommendations for sample-collection, preservation and storage (see Chapter 2); (iii) descriptions of apparatus and equipment with, safety considerations regarding the reagents, standards, and consumable materials; (iv) the use of two columns of different polarities, the so-called primary column and a second confirmatory column and (v) instructions on how to proceed with blank samples, internal standards and surrogate solutions, interferences, calibrations, standardization, and quality control.

It is important to proceed with standards at the nanogram or sub-nanogram levels and to make blank runs in order to avoid any contamination from the system. Quantitative analyses require calibration of the GC system for each analyte in the linear range from the LOD (or better from LOQ) to the highest probable concentration. Some attention should be paid to labile pesticides for which losses can occur in the injector. The use of standards and surrogates is better for quantitative analysis. Each one has a different purpose. The internal standard is added to measure the relative responses of other analytes and surrogates that are components of the solution. The internal standard should be an analyte which is not a sample-component. In contrast, a surrogate analyte is a compound which is extremely unlikely to be found in any

sample, but is added to a sample aliquot before extraction and is measured by the same procedures used to measure other samples. The surrogate is used to monitor the method-performance with each sample component.

Derivatization prior to determination is necessary for involatile and thermally labile pesticides. In some cases, it can be performed to increase the detectability, an example is chlorination prior to using the ECD.

In order to avoid “false positives”, confirmatory techniques are needed. This is usually done by injecting the sample extract onto a second column of different polarity. However, such confirmation does not always constitute a foolproof means of confirmation, and GC-MS is the appropriate approach when available. Even selective detectors can provide misleading information on the nature of the compound. Increasingly, the detection of pesticides will be performed by spectroscopic detectors that allow selective recognition of the separated compounds. Another way of obtaining confirmation using a second column is to apply the so-called two-dimensional capillary GC. Two columns of different selectivity are combined in such a way that the eluate fraction can be transferred directly from one column to another [13]. Another approach for confirmation is the use of chemical derivatization, a technique that has found substantial use when other means of confirmation were not available. It is possible to obtain complementary information on peak-identity by using two (or more) detectors simultaneously. Many routine laboratories applying GC techniques to pesticide analysis use the same GC column and then at the end of the column there is a splitter and the flow is directed to ECD and NPD.

3.2.3. Applications to the main groups of pesticides

The possibilities for GC analysis are reviewed for each main group of pesticides. Attention is paid to those that are not amenable to GC, or require special conditions such as cold injection, or specific derivatization procedures prior to GC. The thermal stability alone is discussed here, and not the storage stability which was discussed in Chapter 2 and which, of course, has to be taken into account in the analysis procedure.

The conventional organochlorine pesticides are not considered, because most of them have been withdrawn and substituted by organophosphorus or carbamate pesticides. Most of them can be analyzed according to the EPA method 508 by GC-ECD, as described in the next section. The chromatographic techniques have been reviewed recently [14].

3.2.3.1. Pyrethroids

They are easily amenable to GC, using a DB-5 or equivalent capillary column and ECD or MS detection [15–17]. An example of their determination in surface water is presented in the next chapter. Figure 4.34 shows the necessity of cleaning the extract before the GC-ECD determination because of the poor selectivity of the ECD with some contaminated surface water samples [15].

3.2.3.2. Organophosphorus compounds

This group is usually analyzed by GC followed by GC, with NPD, after extraction from water. A separation of 38 compounds within this group, using a DB-17 column with flame photometric detector has been presented [18]. Detection limits are in the low $0.1 \mu\text{g/l}$ range using classical off-line extraction procedures from 500 to 1000 ml of drinking-water [19]. However, GC methods are not appropriate for some of these compounds which are thermally labile and/or very polar, such as dichlorvos, temephos, trichlorfon, oxydemeton-methyl or mevinphos [20–22].

The flame photometric detector is more sensitive than NPD and has also been recommended in official methods known as SCA methods (Official Methods of the Department of Environmental Drinking Water Inspectorate Standing Committee of Analysts in the UK). The method utilizes a 25–50 m capillary column of OV-1 or SE-54 coupled with an FPD detector [23].

The atomic emission detection can be very sensitive for this group, using the phosphorus channel. Figure 3.1 gives the chromatogram corresponding to the AED response of an extract from 50 ml of river Meuse water, non-spiked and spiked with several organophosphorus pesticides at the $0.1 \mu\text{g/l}$ level. The system was an on-line solid-phase extraction with gas chromatography and atomic-emission detection [24].

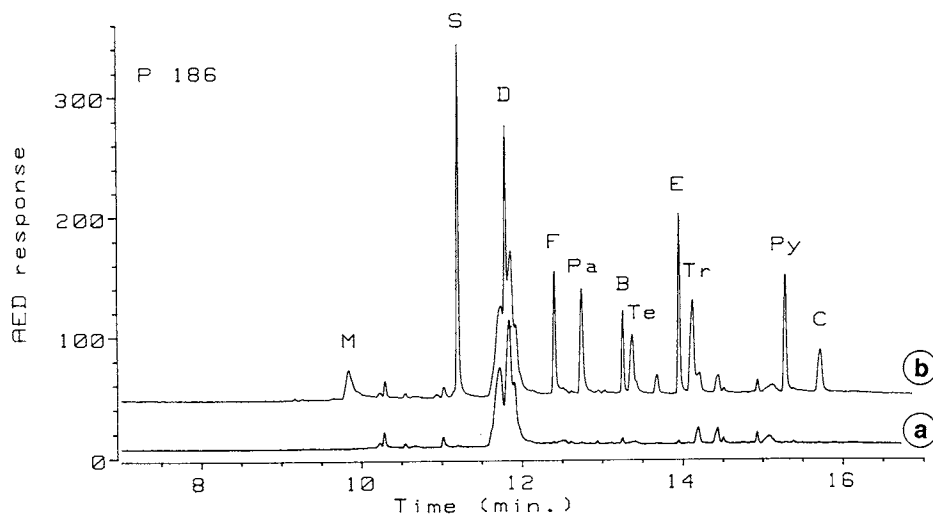


Fig. 3.1. On-line SPE-GC-AED (P channel) of (a) 50 ml Meuse water and (b) 50 ml Meuse water spiked with organophosphorus pesticides at the $0.1 \mu\text{g/l}$ level. GC column, $15 \text{ m} \times 0.32 \text{ mm i.d.}$ SPB-5 (Supelco). Peak assignment: M, mevinphos; S, sulfotep; D, diazinon; F, fenclorphos; Pa, parathion-ethyl; B, bromophos-ethyl; Te, tetrachlorvinphos; E, ethion; Tr, triazophos; Py, pyrazophos; C, couma-phos. From Ref. [24] with permission.

3.2.3.3. *Triazines*

The direct GC analysis of triazines is easy, owing to their sufficient volatility, their moderate polarity and their high response with appropriate detectors. NPD, in its various forms with thermionic or other instrumental configurations, is now the most sensitive and most commonly used detector for triazine analysis. Many examples can be found in the literature and have been reviewed [2,25,26]. ECD can also be applied, but has a lower sensitivity; it is employed either when NPD is not available in the laboratory or when organochlorinated pesticides are analyzed in the same chromatographic run [27]. After an appropriate enrichment step, most of the studies report LODs in the 10–100 ng/l range using GC-NPD. Among the main degradation products, the hydroxytriazine derivatives are not directly amenable to GC, unlike the parent molecules. Although the hydroxy derivatives have been analyzed by GC after derivatization [28], their direct analysis by LC is more appropriate.

3.2.3.4. *Phenylureas*

This group is not easily amenable to GC because some of them suffer rapid thermal decomposition into their isocyanates and aliphatic amines. This problem was overcome by derivatizing the phenylureas to give more stable products or by hydrolyzing them to their corresponding anilines, which were subsequently measured. The first approach was performed using trimethylanilinium hydroxide (TMAH) [[29] or methyl and ethyl iodide [30,31] and subsequent detection by NPD. Heptafluorobutyric acid (HFBA) has also been used for derivatization, with the advantage of using GC-ECD [32,33]. The second approach involved the detection of anilines after derivatization. De Kok et al. have developed a technique for the catalytic hydrolysis of the parent phenylureas and detection of anilines after derivatization with HFBA [34, 35]. The anilines originally present in the sample could be detected in parallel. However, not all the phenylureas could be stabilized by derivatization.

Attempts have also been made to modify the GC conditions for direct injections, especially using cold injection techniques. Grob [36] has optimized the GC procedures with this group of herbicides and has shown that the phenylureas containing a methoxy group on the nitrogen atom, such as monuron and linuron, were among the most stable and could be thus analyzed by cold injection whereas others, such as diuron, metoxuron or neburon, were classified as impossible to analyze using GC.

3.2.3.5. *Carbamates*

The analysis of *N*-methyl carbamates has received more attention than other carbamates because of their greater use. These compounds are not directly GC-amenable because thermal decomposition occurs, to give the respective phenols. Derivatization procedures have been described which led to more stable products and gave an increase in sensitivity when ECD was used [37]. A simple method for the determination of ten thermo-labile carbamates and seven urea-pesticides was described, using derivatization with acetic anhydride followed by GC-MS [38]. As

with phenylureas, attempts have been made to modify the GC conditions, using lower injection temperatures and short columns with NPD [39–41].

3.2.3.6. *Phenoxyalkanoic acids, chlorophenols and other acidic pesticides*

Phenoxyalkanoic acids cannot be analyzed directly by GC because of their acidic character and low volatility. They are usually derivatized to methyl and pentafluorobenzyl (PFB) esters. Methylation is the less laborious method and has been applied to various acidic pesticides; in combination with an appropriate sample enrichment method, detection limits were in the range 0.1–1 $\mu\text{g/l}$ [42]. Diazomethane was also applied [43] and is recommended in the EPA 515.1 Method. Diazomethane is rather toxic and has explosive properties, and moreover, the methyl ester derivatives do not show a high response factor with the ECD. There are some advantages in using pentafluorobenzylation, even if it is more laborious. It can also be applied to chlorophenols which are the important degradation products of phenoxyalkanoic acids. PFB derivatization was applied to chlorophenols and phenoxyalkanoic acids, thus providing sufficient ECD responses [44–46]. Acetylation has been applied to chlorophenols with the advantage of being very simple and is performed directly in water by the addition of acetic anhydride [47]. Derivatization of acidic herbicides using 2-cyanoethyltrimethyl(diethyl)aminosilane (CEDMSDEA) was reported for their determination in water, with the advantage of providing a good response to NPD [48,49].

3.2.3.7. *Other groups*

The GC analysis of sulfonylureas suffers from their polar characteristics and thermal instability. The determination of the decomposition products of chlorsulfuron was described using GC-NPD and characterization by GC-MS [50]. Derivatization was also investigated, as with methylation for chlorsulfuron [51]. Perfluorobenzyl derivatives of chlorsulfuron and metsulfuron-methyl were determined using GC-ECD and were characterized by GC-MS [52].

Acetanilides, i.e., alachlor, metazachlor and metolachlor are directly amenable to GC-ECD or GC-NPD [53]. The GC-NPD of trifluralin, widely used, has also been described.

A recent review dealing with the determination of cereal herbicides residues in environmental samples by gas chromatography provides useful information on other herbicides not discussed above [54].

3.2.4. **Multiresidue analysis**

Multiresidue methods provide a solution to the problem of reducing the cost of analyses while increasing their number. Multiresidue methods cover all the groups of pesticides and require universality of the sample pre-treatment procedure and, as far as possible, of the conditions for the chromatographic separation.

Using GC, unification can be performed for the selection of the column, since many pesticides can be analyzed using a low polarity stationary phase (see Table 3.1). Multidetector can be obtained by the simultaneous connection of the column to two detectors. The limitations of multiresidue analysis come from the derivatization procedures that must be applied to some groups, as seen above, and which are group-specific. In this respect, the EPA methods provide a good guide for the state-of-art in “restricted” GC multiresidue analysis, dividing pesticides into those that can be analyzed directly by GC-NPD or GC-ECD, and those that can be analyzed using the same derivatization reaction and the same detector.

In many cases, derivatization is performed in order to increase the detection response. That is no thermal decomposition occurs, meaning that these compounds are amenable to GC. Therefore, they can be included in multiresidue analyses using GC coupled directly to MS. This is becoming the method for performing GC multiresidue analysis, and is discussed in the last part of this chapter, with examples of relevant applications. Although less often described than MS, the atomic emission detector is also appropriate owing to its ability to monitor several elements simultaneously and selectively. Thirty-four directly GC-amenable chlorinated hydrocarbon pesticides and nitrogen-containing herbicides could be determined at the $0.1 \mu\text{g/l}$ level in extracts from drinking water and from surface water, with simultaneous monitoring of the four elements carbon, sulfur, nitrogen and chlorine [12]. In the same paper, the authors present a compilation of the GC-AED data for more than 400 GC-amenable pesticides under standard conditions (capillary column HP-5, cold PVT-injection with solvent venting, detection using AED) with the corresponding limits of detection in the different AED element traces. The possibilities of GC-AED in pesticide multiresidue analysis, with applications to herbicide analysis in soils, were explored for 181 compounds in another study [55].

3.2.5. EPA methods

The EPA methods are widely implemented in the United States and cover the analysis of pesticides and transformation products included in the US National Pesticide Survey (NPS), which is the most complete list so far for conducting a monitoring programme on pesticides.

The US EPA methods used for analysis of pesticides in water can be divided into three groups: those that use GC with a selective detector, those that use GC-MS, and those that use LC. We discuss here the first group of methods, with emphasis on the GC analytical conditions and detection. The detection limits usually meet the Health Advisory Levels (HAL) from the EPA Office of Ground Water and Drinking Water but, of course, the methods have not been optimized to meet the European regulations at the $0.1 \mu\text{g/l}$ level.

Table 3.3 lists the columns which can be selected, the GC detector, and the derivatization procedure, if any, for various EPA-NPS methods [4–6,56,57]. Not all are

TABLE 3.3
SUMMARY OF THE US EPA-NPS METHODS USING GC TECHNIQUES

EPA Method 507 (NPS Method 1): Determination of nitrogen- and phosphorus-containing pesticides in water by GC-NPD

- Dichloromethane LLE (1 l of water; extract in 5 ml of MTBE)
- GC primary column: 30 m × 0.25 mm i.d. DB-5; confirmatory column: 30 m × 0.25 mm i.d. DB-1701
- Surrogate: 1,3-Dimethyl-2-nitrobenzene
- Intal standard: Triphenyl phosphate
- Compounds detected (LOD in the range 0.1–4.5 µg/l): alachlor, ametryn, ametraton, atrazine, bromacil, butachlor, butylate, carboxin, chlorpropham, cycloate, diazinon, dichlorvos, diphenamid, disulfoton, disulfoton sulfone, disulfoton sulfoxide, EPTC, ethoprop, fenamiphos, fenamirol, fluridone, hexazinone, merphos, methyl paraoxon, metolachlor, metribuzin, mevinphos, molinate, napromide, norflurazon, perbutate, prometon, prometryn, pronamide, propazine, simazine, simetryn, stirofos, tebuthiuron, terbacil, terbufos, terbutryn, triademefon, tricyclazole, vernolate

EPA Method 508 (NPS Method 2): Determination of chlorinated pesticides in ground water by GC-ECD

- Dichloromethane LLE (1 l of water; extract in 5 ml of MTBE)
- GC primary column: 30 m × 0.25 mm i.d. DB-5; confirmatory column: 30 m × 0.25 mm i.d. DB-1701
- Surrogate: 4,4'-Dichlorobiphenyl
- Intal standard: Pentachloronitrobenzene
- Compounds detected (LOD in the range 0.02–0.5 µg/l): aldrin, chlordane, chloroneb, chlorobenzilate, chlorothalonil, DCPA, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, dieldrin, endosulfan I and II, endosulfan sulfate, endrin aldehyde, etridiazole, α -HCH, β -HCH, δ -HCH, γ -HCH, heptachlor, heptachlor-epoxide, hexachlorobenzene, metoxychlor, *cis*-permethrin, *trans*-permethrin, propachlor, trifluralin

EPA Method 515.1 (NPS Method 3): Determination of chlorinated acids in ground water by GC-ECD

- Diethyl ether LLE (1 l of water)
- Derivatization with diazomethane
- GC primary column: 30 m × 0.25 mm i.d. DB-5; confirmatory column: 30 m × 0.25 mm i.d. DB-1701
- Surrogate: 2,4,-Dichlorophenylacetic acid
- Intal standard: 4,4'-Dibromooctafluorobiphenyl
- Main compounds detected (LOD in the range 0.1–1.3 µg/l): acifluoren, bentazone, chloramben, 2,4-D, dalapon, 2,4-DB, DCPA acid metabolites, dicamba, 3,5-dichlorobenzoic acid, dichlorprop, dinoseb, 5-hydroxy diacamba, 4-nitrophenol, pentachlorophenol, picloram, 2,4,5-T, 2,4,5-TP

EPA Method 524.2 (NPS Method 7): Measurement of purgeable organic compounds in water by GC-MS

- Trapping volatile compounds in a tube, heating and desorption
- GC column: 60 m × 0.75 mm i.d. Vocol glass wide-bore with 1.5 µm film thickness or 30 m × 0.53 mm i.d. DB-624 with 3 µm film thickness or 30 m × 0.25 mm i.d. DB-5 with 1.5 µm film thickness
- Surrogate: 4-Bromofluorobenzene and 1,2-dichlorobenzene-*d*₄

- Intal standard: fluorobenzene
- Main compounds detected (LOD in the range 0.02–0.05 $\mu\text{g/l}$) : 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, 1,2-dichloropropane, *cis*-1,3-dichloropropene, *trans*-1,3-dichloropropene

EPA Method 504 (NPS Method 7): Determination of 1,2-dibromoethane, 1,2-dibromo-3-chloropropane in water by microextraction and GC-ECD

- Hexane LLE (35 ml)
- GC primary column: 30 m \times 0.32 mm i.d. Durawax-DX 3 (50% dimethylsilicone and 50% polyethylene glycol); confirmatory column: 30 m \times 0.32 mm i.d. DB-1
- LOD: 0.01 $\mu\text{g/l}$

EPA Method 548: Determination of endothal in drinking-water by aqueous derivatization, liquid-solid extraction and GC-ECD

- Derivatization with pentafluorophenylhydrazine and SPE
- GC primary column: 30 m \times 0.25 mm i.d. SPB-5; confirmatory column: 30 m \times 0.32 mm i.d. DB-1
- Intal standard: Endosulfan
- LOD: 11.5 $\mu\text{g/l}$

EPA Method 525: Determination of organic compounds in drinking-water by liquid-solid extraction and GC-MS

- SPE (11 of water, final extract in 0.5–1 ml of methylene chloride)
- GC column: 30 m \times 0.25 mm i.d. DB-5
- Surrogate: perylene- D_{12}
- Intal standard: acenaphthene- D_{10} , phenanthrene- D_{10} , and chrysene- D_{10}

EPA Method 552: Determination of haloacetic acids in drinking-water by liquid-liquid extraction with MTBE, derivatization with diazomethane and GC-ECD

- MTBE LLE (100 ml)
- Derivatization with diazomethane
- GC primary column: 30 m \times 0.32 mm i.d. DB-1701; confirmatory column: 30 m \times 0.32 mm i.d. DB-210
- LOD: 0.08 $\mu\text{g/l}$ for trichloroacetic acid

NPS Method 6: Determination of ethylene thiourea (ETU) in ground water by GC-NPD

- SPE (50 ml of water, final extract in 5 ml of acetyl acetate)
- GC column: 10 m \times 0.25 mm i.d. DB-Wax; confirmatory column: 5m \times 0.25 mm i.d. DB-1707
- LOD: 9.0 $\mu\text{g/l}$

Adapted from Ref. [6].

multiresidue methods, and some of them are tailored for only one or a few compounds, but are important, for example, for trichloroacetic acid. In general, the EPA methods describe in detail all the parameters for a good monitoring programme, including the storage of samples, the addition of eventual preservation products, and the use of two GC columns except when MS is used. They have the advantage of

including transformation products and are regularly revised in order to include recent developments in analytical methods, or the reduction in the use of organic solvents. At the present, the trends are towards the development of methods including micro liquid-liquid extraction (LLE), more solid-phase extractions (SPE) and the use of GC-MS instrumentation [58,59].

In Europe, the US EPA methods have been modified in order to reduce the detection limits. This can be done by increasing the sample volume, or by reducing the extract volume, which in some methods is originally equal to 5 ml. These two approaches have been performed for the EPA method 507; by extracting up to 4 l of water, with a final extract of 0.2 ml, it was possible to determine molinate, atrazine, simazine and alachlor at concentrations in the range 0.005–0.012 $\mu\text{g/l}$ in the Ebro delta samples [60].

3.2.6. Further trends

The performance of GC for the separation of pesticides comes from the high efficiency of the GC columns and the high sensitivity and selectivity of the common detectors. Many pesticides can be analyzed by this technique. The strongest point is the easy coupling with MS and with other powerful detectors that can complement the structural information given by MS, such as atomic emission, nuclear magnetic resonance (NMR) or Fourier-transformed infrared spectrometry (FT-IR). Another strong point is the increasing availability of better performance GC columns, that can work at high temperatures or with high selectivity, e.g., for enantiomer separation. That makes the technique very powerful; when coupled to automatic injectors and MS it can handle numerous samples and provide a lot of information. Indeed, GC-ECD, GC-NPD and GC-MS are routine methods of analysis for pesticides in water matrices and are currently performed in official laboratories, water companies, and private laboratories. It would be rather difficult nowadays in Western Europe or the USA for a GC-amenable compound in drinking water to escape detection. The technique is very robust, stable, and routinely applicable. The weakest features are the requirement for derivatization of many polar and thermally labile analytes, which thus limits multiresidue determinations.

3.3. LIQUID CHROMATOGRAPHY

Liquid chromatography (LC) separates and detect at room temperatures, generally using methanol–water or acetonitrile–water gradient-eluent mixtures, and is appropriate for the analysis of polar compounds. Therefore, LC is complementary to GC, because it permits the analysis of thermally labile, non-volatile, and highly polar compounds. LC is particularly adapted to multiresidue analysis of pesticides over a wide range of polarity, including their transformation products, without the need to derivatize any compound. LC methods also have a major advantage over GC meth-

ods in that on-line pre- and post-column reaction systems are compatible with the LC. This concept is applied widely for the determination of carbamates. Its increased use was no doubt helped by the recent development of UV diode-array detectors with better sensitivity than similar detectors used a few years ago, so making their use in environmental analysis more attractive. Grosser et al. [5] pointed out that the number of official US EPA methods using LC has grown dramatically to more than 40, and that in searching for most cost-effective methods, the EPA recognized the applicability of LC for non-volatile, thermally labile and polar materials. With the trend towards biodegradable pesticides, which are generally more polar than the old ones, LC is becoming the preferred analytical method for most insecticides and their metabolites, for herbicides and for plant growth regulators. Key target pesticides include carbamates, diquat and paraquat, triazines, phenylureas, phenoxy acids and glyphosate.

Another feature in favour of the development of LC is the ease of automation to couple the sample preparation step using solid-phase extraction (SPE) to the LC system. One difficulty in coupling the SPE of aqueous samples with GC detectors is the need for complete removal of water in the SPE cartridges. GC columns are not compatible with traces of water. This problem does not exist with LC, because most mobile phases are mixtures of water with organic solvents, and automated SPE-LC devices have been available commercially for several years: SPE-GC devices are now just appearing on the market.

The LC-MS coupling is now developing rapidly and is already included in some EPA methods for drinking water and hazardous waste analyses; these are discussed in the second half of this chapter.

3.3.1. Selection of the analytical conditions

3.3.1.1. Column characteristics

LC has a variety of separation modes and mobile phases for optimizing a separation. In GC the separation mechanism is based on the interactions between the stationary phase and the solute. In LC the separation mechanisms appear more complicated because of the many primary and secondary interactions between the stationary phase, the mobile phase, and the solute.

Stationary phases are usually classified according to the separation mechanisms. To a first approximation these are: (i) adsorption chromatography using silica, alumina, or silica modified by polar groups; (ii) reverse-phase chromatography, using alkyl- or phenyl-bonded silicas, apolar copolymers, or carbonaceous sorbents; (iii) ion-exchange chromatography using sorbents containing ion-exchanger groups at their surface and (iv) size-exclusion chromatography.

In practice, reversed-phase chromatography (RPLC) with alkyl-bonded silica is by far the most popular separation mode in all the fields of application; it is estimated that more than 80% of the published LC separations use it. RPLC is used al-

most exclusively in all EPA LC methods, with the exception of ion chromatography which is used for glyphosate and sulfonic acids. There are several reasons for this situation. First, reversed-phase materials, especially *n*-octyl- and *n*-octadecyl-silica (C_{18}) columns can be applied widely, with a simple gradient of mobile phase from pure water to pure methanol or acetonitrile, and compounds having a wide range of polarity can be separated. The columns have good characteristics: high mechanical stability, long life-times, and batch-to-batch reproducibility; they equilibrate rapidly when the composition of the mobile phase is changed. The elution order can be roughly predicted from the hydrophobicity of the solute, as indicated by its water-octanol partition constant, values of which were given in Chapter 1 for many pesticides. Therefore, the most commonly used LC columns in environmental analysis of pesticides are 25×0.46 mm i.d., packed with $5\text{--}10\text{ }\mu\text{m}$ C_{18} silica particles. This length ensures a sufficient efficiency of the column for separating a large number of solutes in one run.

Alkyl-silicas are obtained by the chemical bonding of an alkyl chlorosilane onto the silanol groups of the silica. Therefore, the number of bonded alkyl chains will depend on the specific surface area of the silica: this explains why C_{18} silica prepared from silicas having different characteristics can have different carbon contents, which reflect the number of grafted alkyl chains. After bonding, some silanol groups are still present at the silica surface, especially if an alkyl-, di- or tri-chlorosilane was used for the synthesis. In order to avoid their effects on some polar compounds, C_{18} silicas are often "end-capped", meaning that a final bonding has been applied to the free hydroxyl groups using chloro-trimethyl silane. Several C_{18} silicas are therefore available with a range of characteristics. Generally, however, apart from C_{18} silicas with very low carbon contents, there are only small differences between the various conventional C_{18} silicas, and it should not be too difficult to adapt the analytical conditions to reproduce a published separation using a material different from that available in the laboratory. Some peak inversion can be observed between columns, but with no great changes in retention times for the same analyte. The commercial catalogues list many applications, sometimes using the term " C_{18} silica specific for environmental analysis" for the separation of pesticides, with no mention of this specificity. The practitioner will find there many examples of what can be achieved in LC pesticide separations. Some columns are better adapted to the separation of acidic compounds, or of basic compounds, respectively, that peak-tailing is reduced for the corresponding analytes as a result of a specific end-capping procedure.

Ionic compounds are not well retained by conventional C_{18} silicas with the usual reversed-phase mobile phases. Some pesticides, such as diquat or paraquat, are ionized, or are ionizable and it is then necessary to use ion-exchange chromatography with ion-exchanger stationary phases, or C_{18} silica operating in the ion-pairing mode when a counter-ion is added in the mobile phase.

There is currently some discussion about the use of shorter, narrow-bore columns

(2 mm i.d. or less) to reduce solvent usage. When packed with the same materials they have the same resolving power and are more sensitive for the same injected volume, as standard columns (4–5 mm i.d.). However, narrow-bore columns make higher demands on the equipment, which explains their slow introduction into laboratories. However, we can expect further development in the near future, owing to their easy connection to MS detectors and to the increase of LC-MS.

3.3.1.2. *Mobile phases*

The composition of the mobile phase chosen for a separation depends on both the analytes to be separated and the detection mode.

The separation of neutral pesticides is performed using a gradient composition of mobile phase with water and an appropriate organic solvent. The most commonly used organic solvents are methanol and acetonitrile. A third organic solvent, such as tetrahydrofuran, can be included, but usually in low content. Because of secondary interactions with the organic solvent, the retention order can be slightly different if a water–methanol or a water–acetonitrile gradient is applied. The two solvents are sometimes used in combination with water. A salt, sometimes improperly named a “buffer”, is often added to the aqueous solvent and, although neutral analytes are not affected by the salt, its addition can improve the peak shape of some analyte as a result of solubility effects.

Some pesticides and/or transformation products have acidic or basic properties which must be known in order to select the pH of the mobile phase so that the analytes are in their neutral form, i.e., when possible, two units above the pK_a values for basic analytes and two units below the pK_a for acidic pesticides.

The quality of the water used, of the organic solvent, and of any added chemicals is very important in trace-level detection, especially when the UV detector is used. Solvents with low absorption properties at low wavelengths (RPLC-grade solvents) are available. An increase in the amount of organic solvent in the gradient should not induce a large drift of the base line, which would prevent the use of the detector at its lower sensitivity. When detection is carried out at low wavelength (<220 nm) acetonitrile is preferred over methanol. The water should not contain impurities that would be concentrated at the beginning of the gradient when the water content is high and then be eluted at the end of the gradient as peaks which interfere with those of the samples. This can easily be checked by recording a solvent gradient blank. When only a reproducible drift is observed, which can be explained by the difference of absorbance between water and the organic solvent, this can be recorded and subtracted for real sample analysis.

There are also some limitations in using MS for detection, where not all salts can be used. This is discussed further in the second part of this chapter.

3.3.1.3. *Sample introduction*

Once the sample has been prepared, the extract is introduced into the column via

an appropriate injector. The universal injector is the typical six-port injection valve which permits the injection of reproducible volumes without stopping the mobile-phase flow. When the valve is in the "load" position the sample is introduced into a "sample loop" via a syringe, or via aspiration by vacuum in some automated injection systems. Then the valve is rapidly switched to the "inject" position and the mobile phase flushes the loop's contents onto the column. A minimum fitting is required between the injector and the column in order to reduce the length of diffusion of the loop's contents into the column. Sample volumes can be from 0.1 μl to 100 ml, depending on the sample loop, the design of the valve, and the dimensions of the analytical column.

In trace-analysis using a standard analytical column (15–25 \times 4–5 mm i.d.), volumes of sample extract in the range 10–200 μl are usually injected by completely filling the sample loop to ensure reproducibility of the injected volume. Because reproducible volumes are injected, in contrast to GC, it is not necessary to systematically use an internal standard. Very often, external standardization is performed for all the sample components by separate injection of standard solutions through the same loop. It is important to use the same sample loop, because sample-loop volumes are only within 15–20% by the manufacturers.

Another important point, which can be the source of many practical errors, is the effect of the composition of the injected solution. The solution in which the extract is dissolved should be chosen with care for the initial composition of the gradient applied for the separation of the analytes. When the extract is dissolved in a pure organic solvent and the initial composition of the mobile phase contains a large amount of water, the injection of a 100–200 μl volume will cause distortion of the first eluted peaks, usually giving double peaks. This is easily explained by the fact that the introduction at the head of the column of a volume of 100–200 μl will cause the elution to begin with a stronger elution power than the initial mobile phase. This effect does not occur with a smaller injected volume, up to 5–10 μl , or when the initial mobile phase contains a large amount of organic solvent. Therefore, one is recommended to dilute the extract with the initial mobile phase, or, if there is insufficient dissolution, to use a minimum of organic solvent added to the initial mobile phase.

3.3.1.4. *Common LC detectors (except MS)*

The important parameters for characterizing LC detectors are the same as we have given for GC detectors, i.e., sensitivity, selectivity, linearity and the possibility of providing qualitative information. The LOD is specific to the detector and the analyte of interest, but is also influenced by the analytical conditions, i.e., the composition of the mobile phases, oxygen content, injection system, efficiency of the analytical column, variations of the temperature, etc. There is the same difference as given for GC detectors, between the LOD, defined as two or three times the noise level, and the LOQ, which is 5–10 times higher.

Commonly used LC detectors in pesticide analysis are UV, fluorescence, electro-

chemical and MS. The selectivity of the UV detector depends greatly on the selection of the wavelength but, in general, it is lower than that of fluorescence and electrochemical detectors. Linearity of response of a UV detector can be obtained over a range of five orders of magnitude, whereas those of fluorescence and electrochemical detectors cover a range of one to three orders of magnitude.

3.3.1.4.1. UV Detector and diode-array detector. Detector technology has improved dramatically in recent years and considerable improvements in detection limits have been achieved. Single-wavelength detectors are very sensitive, with LOD at the low nanogram level for some pesticides with high molar absorptivity. The introduction of the diode-array UV (and visible) detector (UV DAD), which contains an array of more than 200 photodiodes which can simultaneously monitor the UV(-Vis) spectra, allows the collection of spectra from fast eluting peaks. The DAD software permits automated matching of the sample peak against known standards previously introduced into its "library". The purity of a peak can be assessed by comparing the spectra collected at the beginning and end of each peak. The UV DAD has become the basic environmental LC detector. The comparison of the diode array's full spectra provides results with a good confidence level and is a great help in identifying the components, especially those with characteristic spectra. Peak purity information is also very useful for quantitative results.

Table 3.4 gives the UV characteristics of some priority pesticides and their transformation products.

The major problem encountered with UV DAD is its lack of selectivity when extracts from complex matrices are analyzed at common wavelengths. Some examples, presented in the next chapter, show the need of an additional clean-up or an optimization of the sample pre-treatment when contaminated surface water samples are analyzed. The detection limits depend more on the isolation and extraction procedures selected than on the characteristics of the LC-UV DAD method.

3.3.1.4.2. Fluorescence detector. The fluorescence detector (FLD) is very selective and sensitive, since it is based on the measurement of the emitted light, and only a few molecules can re-emit part of the absorbed light in the form of higher wavelength light. Degassing the mobile phase is recommended for avoiding oxygen-quenching. The detector's excitation and emission wavelengths can be selected independently. They can be programmed as a function of time, and of the compound to be detected. Pollutants such as polycyclic aromatic hydrocarbons are fluorescent and have been used extensively in the development of FLDs which can detect compounds at the picogram levels. Their extreme sensitivity is the basis of the development of pre-column and post-columns reactors which convert non-fluorescent compounds into fluorescent derivatives. The most relevant example in pesticide analysis is the on-line automated post-column reactor for the detection of *N*-methylcarbamates and glyphosate.

TABLE 3.4
UV CHARACTERIZATION OF PRIORITY PESTICIDES AND THEIR TRANSFORMATION
PRODUCTS: THE MAXIMUM ABSORPTION WAVELENGTH (nm) IS GIVEN FIRST (FROM REF.
[4] AND LABORATORY-MEASUREMENTS)

Acetochlor	<200	Fluazipop	225
Acifluorfen	<200	Fluometuron	240
Alachlor	<200	Fluroxypyr	211
Aldicarb	207, 220, 247	Flusilazole	213
Ametryn	220	Folpet	225
Amitrole	<200	Glufosinate (amm.)	<200
Anilazine	254	Glyphosate	<200
Atrazine	223, 268	Hexaconazole	219
Azinphos-ethyl	300, 230	Hexazinone	252
Azinphos-methyl	300, 229	Imazapyr	260
Benalaxyl	262	Ioxynil	235, 280
Benazolin	215, 250	Iprodione	206
Bendiocarb	277	Isoproturon	243
Benomyl	279, 235	Linuron	242, 280
Bentazone	220, 232, 316	MCPA	230, 280
Bifenox	300, 201	MCPB	230, 280
Bromacil	210, 277	Mecoprop	230, 282
Bromoxynil	218, 254	Metam	208, 232
Bupirimate	234, 306	Metamitron	306, 243
Butocarboxim	238, 280	Metazachlor	<200, 220
Butachlor	<200	Methabenzthiazuron	269, 223
Butoxycarboxim	<200, 238, 280	Methiocarb	<200, 225, 254, 265
Butylate	230	Methomyl	232
Captan	<200	Methoxychlor	<200, 240
Carbaryl	219, 270	Metobromuron	244
Carbendazim	223, 280	Metolachlor	<200, 214
Carbetamide	236	Metoxuron	245, 211, 293
Carbofuran	280, 225	Metribuzin	295, 230
Chloramben	215, 302	Metsulfuron	230, 277
Chlorfenvinphos	205, 247	Molinate	208
Chloridazon	229, 284	Monolinuron	244, 280
Chlorothalonil	229, 254	Monuron	246
Chlorotoluron	244, 211	Napropamide	214
Chlorpropham	237, 211	Neburon	246
Chlorpyrifos	289, 230	Norflurazon	239
Chlorpyrifos-methyl	289	Oxamyl	216
Chlorsulfuron	232, 276	Paraquat	256
Chlorthiamid	276, 216	Parathion	280
Clofentezine	278	Parathion-methyl	280
Clopyralid	285	Pebulate	230
Cyanazine	224, 268	Pendimethalin	238, 288
Cycloate	230	Permethrin	271
Cymoxanil	264	Phenmedipham	234, 270
Cypermethrin	<200, 230, 270	Picloram	220

2,4-D	208, 244	Pirimicarb	244, 308
Dalapon	<200	Pirimiphos-ethyl	240, 300
2,4-DB	208	Prochloraz	204, 224
DDT	<200, 233	Prometon	219, 244
Deltamethrin	203, 223, 278	Prometryn	223, 254
Desmetryn	238	Propachlor	<200
Diazinon	248, 288	Propanil	250
Dicamba	277	Propazine	220, 260
Dichlobenil	290	Propham	236
Dichlorprop	208, 228, 285	Propyzamide	204, 235
Dichlorvos	<200	Simazine	223, 244, 263
Diiflubenzuron	258	Simetryn	224
Dinoseb	269, 21	Tebuconazole	<200, 220
Dimethoate	<200	Temephos	<200, 250
Dinoterb	276, 310	2,4,5-T	214
Diquat	310	Terbacil	278, 220
Diuron	252, 211	Terbumeton	220, 254
DNOC	271	Terbuthylazine	225
EPTC	229	Terbutryn	225, 261
Ethiofencarb	<200	Thiabendazole	300, 240
Ethirimol	270, 230	Thiobencarb	<200, 225
Ethofumesate	224	Tri-allate	210
Etridiazole	234	Trietazine	230, 265
Fenamiphos	248	Trifluralin	276, 233
Fenoxaprop-P	237, 280	Vernolate	228
Fenoxycarb	230, 281	Vinclozin	<200, 222
Fenuron	241		

3.3.1.4.3. Electrochemical detector. Compounds which contain functional groups which are readily oxidized or reduced (mainly phenols, amines, nitro aromatics, aldehydes, and ketones) can be determined using the electrochemical detector (named here ED, and not ECD as is usually done, in order to avoid confusion with the electron capture detector). Improvements in the technology have been achieved so that the problems associated with the first generation of electrochemical detectors have been solved by the introduction of automatic, self-cleaning electrodes. However, the ED still has the reputation of being difficult to handle and for having a limited stability. It is widely used in clinical chemistry and for routine analysis, one example being in the analysis of catecholamines.

By the careful selection of the potential applied to the electrodes, both the selectivity and the detection limits can be optimized. Electrochemical detectors can be very sensitive, with LOD in the low picogram range. Coupling on-line with the UV DAD can be used for confirmation of electro-active analytes.

3.3.1.4.4. Other detectors. The refractive-index detector is not used for environmental applications because its sensitivity is too low and it is incompatible with solvent gradients.

Among other detectors, the light-diffusion detector (LDD) has been shown to be good for the detection of compounds which do not have any chromophores in their structure. Valuable applications can be found in lipid or amino acid analysis, but are not often described for pesticide analysis.

Over the years, the use of GC detectors in LC has been a continual challenge. Vaporization interfaces are required to connect the outlet with the GC detector. In most published work, they consist of a transfer tube mounted in an oven or in a massive stainless steel block heated at 300°C [61]. Thermionic detection has also been coupled to packed microcapillary columns by a nebulization interface with a mobile flow rate of 5 $\mu\text{l}/\text{min}$ [62]. Although interesting applications have been described in the literature, the routine use of these techniques should require available equipment.

3.3.1.5. *Post-column reaction*

The weakest part of an LC system is still the detector because of the limited possibilities of UV/Vis fluorescence and electrochemical detectors. It is possible to detect compounds at very low concentrations only if they possess a specific chromophore, or fluorophore, or electro active group, respectively. Consequently, chemical methods are of interest for increasing the sensitivity of detection by the addition of a new chromophore or fluorophore by chemical reaction. Another goal is to achieve an increase in selectivity by applying a specific and selective derivatization reaction which will derivatize only the compounds of interest and allow them to be detected selectively in complex matrices. The addition of fluorescent properties to the molecules is particularly beneficial owing to the sensitivity of the fluorescence detection. The commonly used fluorescent agents are phenyl isocyanate which can be added to alcohols, *o*-phthalaldehyde (OPA) to be added to primary amines, and 9-fluoro methyl chloroformate (FMOC) to be added to primary and secondary amines.

Post-column reaction should preferably be done on-line for maximum accuracy, but requires additional equipment such as pumps to add reagents, and eventual mixing and heating devices and safety valves to control back pressure in the system. The advantages are that the analytes are separated in their original form, which often permits the adoption of published separations. Artefacts play a minor role, as opposed to the situation when the derivatization is done prior to the separation step. Moreover, the reaction does not need to be complete, and the reaction products need not be stable. The only requirement is reproducibility. Commercial post-column derivatization equipment (Pickering Laboratories) is available for the analysis of carbamates and glyphosate.

3.3.2. **General considerations about analytical procedures**

The general considerations which were given for a GC analysis are valuable for LC analyses and are not reconsidered now.

We have pointed out that internal standards are not compulsory with commonly

used UV detectors, because of the large difference in response which exists between compounds and also because it is easy to achieve reproducibility of the injected volume. Therefore external standardization is preferred. However, the use of a surrogate is recommended. In the case of LC-MS, and because of the variability of response during the analysis time, one is recommended to use standard or labelled analytes: external calibration is still very commonly used, especially with automated on-line SPE coupling.

Regarding the use of a confirmatory column, there is not too much choice when operating in the reversed-phase chromatography mode with C₁₈ silica columns. Although some EPA methods recommend the use of an extra C₁₈ silica column providing different retention times, it is better to select a confirmatory column operating on the basis of a different retention mechanism and which will induce inversion of retention times. One solution is to use a cyanopropyl-modified silica which can operate in both normal and reversed-phase modes (the so-called mixed mode).

3.3.3. Applications to the main groups of pesticides

In principle, all compounds are amenable to LC, but not all are directly and easily detectable by the usual detectors. In practice, pesticides such as the organochlorines, some acetanilides, and pyrethroids should not be analyzed by LC, because they are easily analyzed by GC-ECD or GC-NPD. The complementarity of the methods should be exploited to the maximum. As a general rule, pesticides which are directly GC-amenable are better analyzed by GC, although there is an interest in including them in LC multiresidue analysis containing other non-GC-amenable pesticides or when the detection limits are competing; one example of this is in the determination of chlorotriazines.

3.3.3.1. Organophosphorus

The LC analysis of this group is encountered when some thermolabile and/or polar compounds such as dichlorvos, temephos, trichlorfon or oxydemethon-methyl are included in the list of organophosphorus pesticides to be determined. The organophosphorus pesticides of the so-called parathion group, with aromatic moieties, do not represent a problem in UV since they contain good chromophores. Problems arise with pesticides which have weak chromophores or none, such as like malathion or trichlorfon. In any case, the DAD detector has been applied routinely to the trace-determination of many organophosphorus pesticides in water samples [20,22].

3.3.3.2. Triazines

Triazines can be analyzed directly by GC-NPD and are also easily analyzed by LC-UV DAD. Owing to their strong absorbance at 220 nm, the detection limits obtained by the two procedures are roughly equivalent, although a lower sensitivity was reported in LC-UV than GC-NPD [63]. Many examples can be found in the lit-

erature [2,3,26]. Detection limits below $0.1 \mu\text{g/l}$ are frequently reported for drinking water samples after an enrichment step using either off-line or on-line procedures. Examples of multiresidue analyses including the triazine and phenylurea herbicides are shown in Chapter 4 (see Fig. 4.13). The advantage of LC is that the hydroxy derivatives can be included with the other transformation products [59]. The identification of the hydroxy derivatives using UV DAD is easy because of the characteristics of their UV spectra.

In attempts to increase the selectivity of the detection, investigations have been performed on the use of the electrochemical detectors, but without any real increase [65–67].

3.3.3.3. *Phenylureas*

All urea herbicides and their degradation products can be determined by LC-UV. Linuron and three metabolites could be determined simultaneously [68]. After off-line or on-line enrichment steps, the detection limits in drinking-water are in the low $0.1 \mu\text{g/l}$ range using simple UV DAD (see Fig. 4.13) [69]. Phenylureas have well defined spectra with maxima around 244 nm, so that UV DAD can give spectral confirmation. With contaminated surface water samples, a clean-up step should be applied to reach such low detection levels for the more polar phenylureas such as metoxuron [70].

Phenylureas are easily detected by electrochemical detection and the coupling of the ED to the UV DAD increases the selectivity and reinforces the identification of compounds [71–74]. Nielen et al. applied LC-ED to surface water samples and found it to provide sensitive screening, with no clean-up of samples, at the sub $\mu\text{g/l}$ level [75]. Electrode contamination could be suppressed by pulsing the electrode periodically to high potentials. An example is given in Fig. 3.2, representing the analysis of phenylureas and triazines in a sample of contaminated surface water. It is clear that the amount of interfering compounds is lower in the ED than in the UV chromatogram and, at the potential applied to the carbon electrode, very few compounds apart from phenylureas are electroactive. Although the sensitivity is not really increased in comparison to UV detection, the selectivity is increased with the ED.

Discrimination between phenylureas and the substituted anilines, which were reported as their main degradation products, can be achieved by optimization of the LC separation. The discrimination has also been performed by a combination of GC and LC techniques [35] or by an appropriate sample pretreatment which removed the anilines [76].

Other attempts have been made to increase the sensitivity by derivatization and using a selective detector. A hydrolysis was performed on metoxuron and the product converted into a fluorescent derivative by using dansyl chloride [77]. Another derivatization procedure was described which involved the insertion of a module for photodegradation of the phenylureas between the LC column and the fluorescent detector, and by derivatizing the photodegradation products with *o*-phthalaldehyde [78].

The electron-capture detector was used in the analysis of phenylureas herbicides after their derivatization with HFBA [79]. Packed-capillary reversed-phase liquid chromatography was coupled with ECD and showed that linuron could be determined at the sub $\mu\text{g/l}$ level in surface water [80].

3.3.3.4. Carbamates

The LC analysis of this group of compounds is preferred because of their thermal degradation. However, not all the compounds can be detected by UV. Those containing a UV chromophore, such as carbofuran, carbaryl, benomyl, and carbendazim, can be detected directly by UV or UV-DAD after an off-line or on-line enrichment step [69,81]. Other compounds could be determined directly using electrochemical [82–85] or fluorescence detectors [86,87]. A large increase in sensitivity was obtained by using fluorescence. The electrochemical detection was reported for eight carbamates, using a flow cell with a wax-impregnated graphite electrode working at positive potentials [83]. Detection limits were below 5 ng. Andersen et al. [84,85] used a Kel-F-graphite electrode for the direct determination of carbamates in water and were able to obtain detection limits in the picogram range. In order to avoid the accumulation of reaction products at the surface of the electrode, the authors apply an in situ cleaning by pulsing the electrode periodically to extreme potentials. By using a platinum working electrode in another study it was reported that detection limits in the nanogram to the picogram range could be reached, depending on the compound and on the potential applied [88].

The number of carbamates with direct UV, electroactive or fluorescence properties is limited, and for the others a precolumn or post-column derivatization is needed. Derivatization methods applied to carbamates have been reviewed recently [89,90]. A precolumn mode was described by Lawrence and Leduc [91]. The carbamates were hydrolyzed to phenols which could react with dansyl chloride to produce dansyl derivatives which were then separated and detected. The post-column mode was introduced by Moye et al. [92] and has been widely recognized for its sensitivity and selectivity; an automated on-line derivatization device is commercially available, and is the basis of the official EPA Method 531.1. The *N*-methylcarbamates are separated on a C_{18} silica column and are also hydrolyzed to methylamine and phenols; the methylamine is then derivatized with OPA or with a mixture of OPA with 2-mercaptoethanol (OPA/MERC) in order to produce highly fluorescent 1-hydroxy ethylthio-2-methylisindole (see Fig. 3.3 for the reaction scheme).

The method above has been improved and provides detection limits of 0.1–0.5 ng [93]. These detection limits can be obtained routinely with the Pickering post-column derivatization instruments. This method is very selective since only *N*-methylcarbamates and *N*-methylcarbamoyloximes are then detected. The high selectivity and sensitivity is illustrated in Fig. 3.4. for the multiresidue analysis of a mixture of 44 pesticides, including several carbamates, using a C_{18} silica column for the separation and on-line detection with UV DAD and the Pickering Laboratories Car-

bamate Post-Column Derivatization Instrument coupled with fluorescence. No compounds other than carbamates are detected by fluorescence, showing the selectivity, and there is 40-fold factor in difference in sensitivity between the UV- and the FLD chromatograms. Detection limits measured by a signal-to-noise ratio of 5 are below 10 pg injected amounts (injected volume 10 μ l) for carbamates with the experimental conditions of Fig. 3.4.

The use of a solid phase reactor containing an anion exchanger for the hydrolysis of the *N*-methyl carbamates has simplified the post-column derivatization system

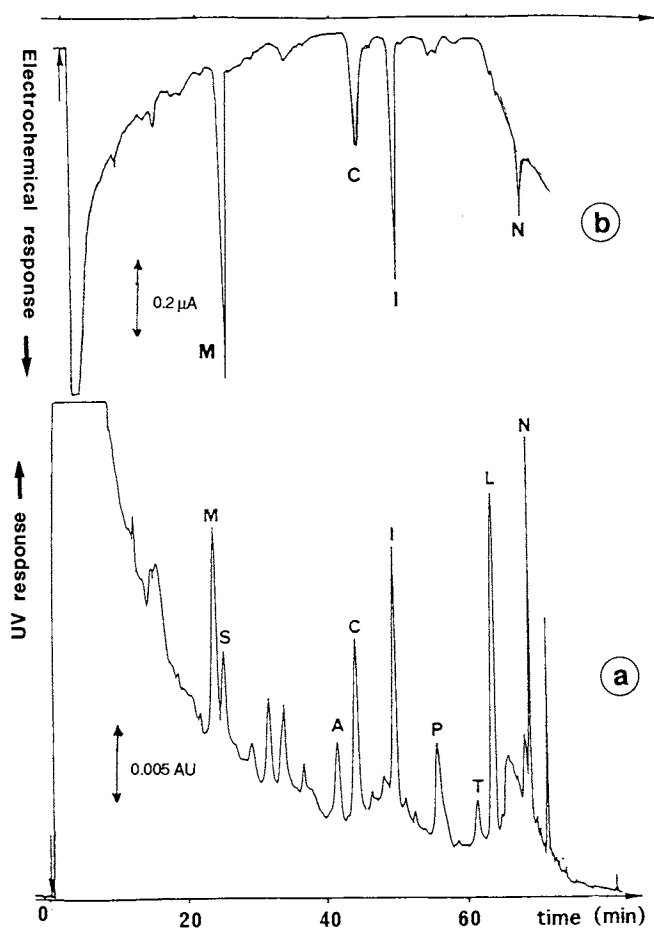


Fig. 3.2. UV and electrochemical signals obtained from the analysis of a 500 ml sample of surface water (River Rhone) spiked with a mixture containing phenylureas and triazines, each one with 1 μ g/l. Analytical column, 250 \times 4.6 mm, Nucleosil C₁₈, 5 μ m; mobile phase, water-acetic acid and potassium acetate, 0.1 M, pH 4.6) and acetonitrile gradient. (a) UV detection at 254 nm; (b) electrochemical detection at 0.8 V (versus Ag/AgCl). Solutes: M, metoxuron; C, chlorotoluron; I, isoproturon; L, linuron; S, simazine; A, atrazine; T, terbutylazine.

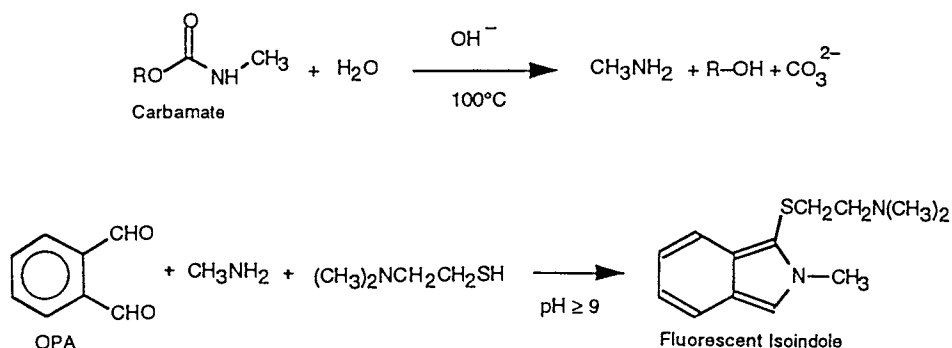


Fig. 3.3. Hydrolysis of carbamates and derivatization reaction involved in the Pickering Laboratories Carbamate Post-Column Derivatization Instrument.

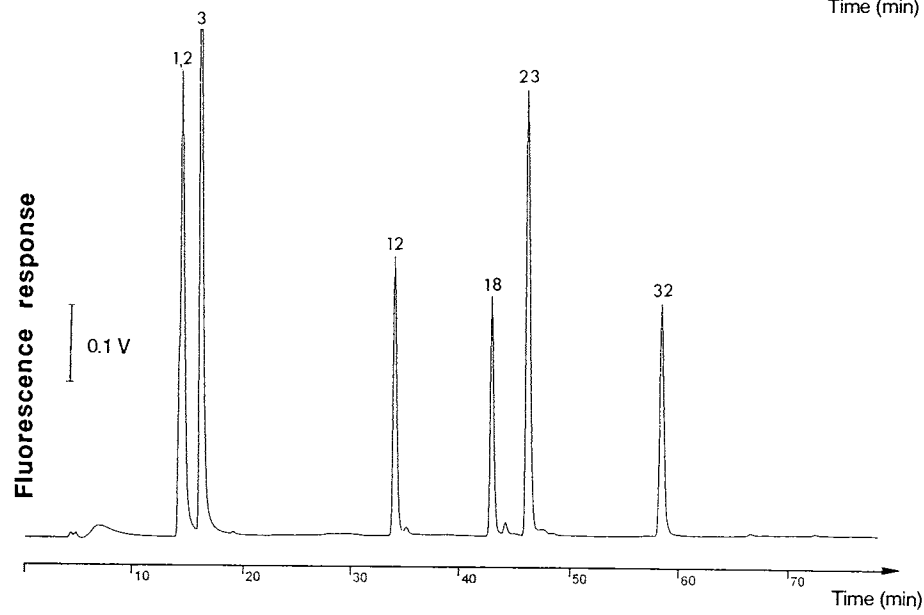
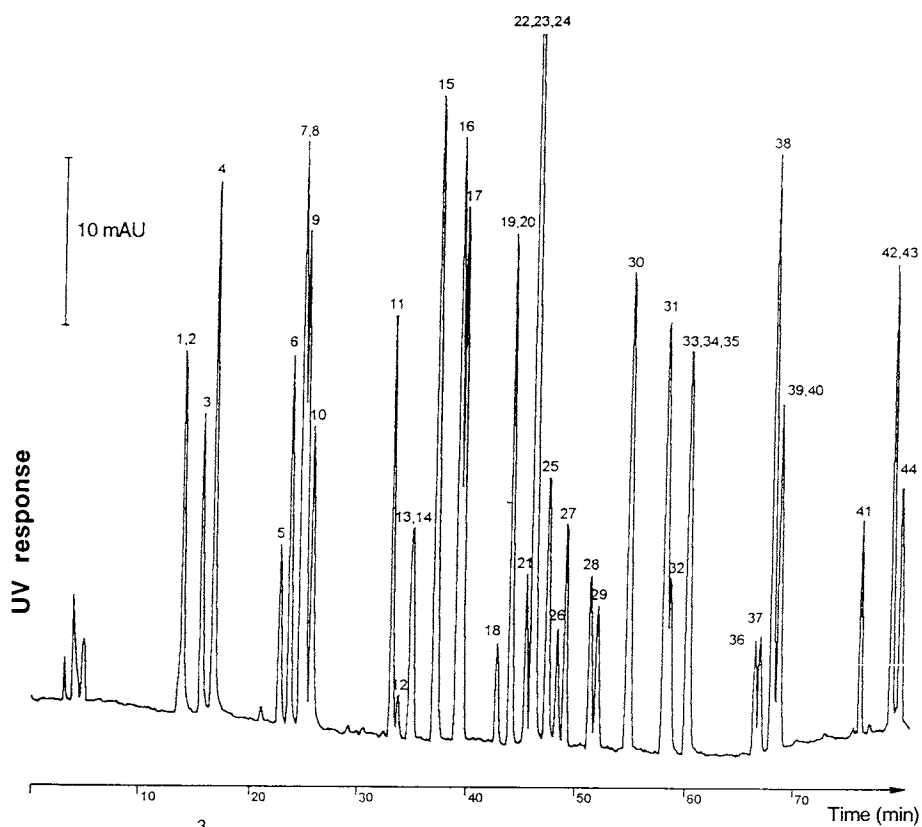
[94,95]. Post-column solid-phase hydrolysis was applied to the analysis of a large number of *N*-methyl carbamates and their sulfone and sulfoxide degradation products, using an off-line solid-phase extraction from 50 ml samples, with detection of compounds at levels below 30 ng/l [96]. The procedure was automated with an on-line preconcentration, with the same detection levels from 5 ml samples; the chromatogram corresponding to the analysis of a 5 ml surface water sample is presented in Chapter 5 (Fig. 5.19) [97].

A post-reaction detector was also developed for the determination of the fungicides thiram and disulfiram [98]. Detection was based on a post-column complexation of these analytes on a solid-state reactor packed with finely divided metallic copper to form a coloured copper complex, copper(II) *N,N*-dimethyldithiocarbamate, with an absorption maximum at 435 nm.

3.3.3.5. Phenoxyalkanoic acids, chlorophenols and other acidic pesticides

LC has been used in order to avoid the derivatization procedure necessary for GC. The simplest method is based on an enrichment step, a reversed-phase separation, and direct UV DAD detection, since phenoxyalkanoic acids have characteristic UV spectra with two maxima, the first at 230–235 nm and a second, less intense, at 280 nm [99,100]. The second wavelength was selected because many fewer interfering compounds are detected at 280 nm than 230 nm [101]. The LC determination of phenols and derivatives, which are the main degradation products, has been reviewed recently [102]. Chlorophenols can be detected at 220 nm or 280 nm, as for phenoxyalkanoic acids.

In order to avoid the number of interferences detected by UV, some more selective detection modes have been investigated. Simple indirect photometric detection at 510 nm was described, with the addition of 1,10-phenanthroline in the mobile phase [103]. Electrochemical detection was also tested for the detection of chlorophenols [104,105]. The selectivity was effectively increased, with a lower back-



ground, but the sensitivity was no better than obtained with UV. For the lower chlorophenols, a post-column photo-conversion to phenol by UV irradiation, followed by fluorescence detection, was described and was found to be very selective when applied to surface water-samples [106]. Precolumn derivatization has also been described as enhancing the selectivity and/or sensitivity of determination of phenol and chlorophenols [107,108].

For phenoxyalkanoic acids, selectivity is usually provided by the sample-handling step, taking into account the fact that these compounds can be ionized (see the next Chapter) [100,109,110].

3.3.3.6. Highly polar pesticides (quats, glyphosate, aminotriazoles, etc.)

3.3.3.6.1. Glyphosate. Glyphosate is a widely used, very broad-spectrum non-selective post-emergence herbicide. Its trace-determination and that of its metabolite, aminomethyl phosphonic acid (AMPA) is difficult because it is highly soluble in water, and insoluble in organic solvents. Although the GC analysis of glyphosate has been described, through derivatization with the reagent *N*-methyl-*N*-*t*-butyl dimethylsilyl)trifluoroacetamide in dimethylformamide [111], or through derivatization with pentafluoropropionic anhydride plus trifluoroethanol [112], the characteristics of glyphosate and AMPA make them more easily analyzed by LC. Since the molecules do not possess a chromophore, they need to be derivatized for detection. Both pre- and post-column derivatization have been developed. Derivatization in the precolumn procedures is carried out with 9-fluorenyl-methyloxycarbonyl chloroformate (Fmoc-Cl), yielding highly fluorescent derivatives. This method was recently applied by Sancho et al. [113] to soil samples. After extraction in alkaline media, neutralization, and derivatization, two different LC methods have been applied. The first is focused on the determination of glyphosate only and requires a liquid-liquid extraction step with ethyl acetate to remove the excess of reagent prior to RPLC analysis with a C₁₈ analytical column under isocratic elution conditions. The second method makes use of coupled-column LC for on-line sample clean-up of diluted ex-

Fig. 3.4. Multiresidue analysis of a mixture containing 44 pesticides and degradation products with UV diode array detection and on-line post-column reactor and fluorescence detection. Analytical column, TSK ODS-80TM, 250 × 4.6 mm, 5 μm; mobile phase, acetonitrile gradient with phosphate buffer, 0.05 M at pH 7.2; flow rate, 1 ml/min; column temperature, 30°C. UV diode-array detection at 220 nm. Attenuation range, 108. Post-column reactor at 100°C, flow rate of NaOH and OPA/MERC, 0.3 ml/min; Fluorescence detector, attenuation range, 4489. Solutes: 1, aldicarb sulfone; 2, oxamyl; 3, methomyl; 4, de-isopropylatrazine; 5, metamitron; 6, hydroxyatrazine; 7, ioxynil; 8, de-ethylatrazine; 9, chloridazon; 10, carbendazime; 11, hydroxyterbutylazine; 12, aldicarb; 13, carbetamide; 14, metoxuron; 15, simazine; 16, cyanazine; 17, de-ethylterbutylazine; 18, carbofuran; 19, methabenzthiazuron; 20, pirimicarb; 21, chlortoluron; 22, atrazine; 23, carbaryl; 24, thiofanox; 25, thiram; 26, isoproturon; 27, diuron; 28, propachlor; 29, propham; 30, propazine; 31, terbutylazine; 32, methiocarb; 33, linuron; 34, phenmedipham; 35, desmedipham; 36, chlorpropham; 37, tebuconazole; 38, folpet; 39, alachlor; 40, flusilazole; 41, fenoxaprop; 42, pendimethalin; 43, trifluralin; 44, triallate.

tracts (this method is discussed in Chapter 5) and allows the determination of both glyphosate and AMPA. Other pre-derivatization agents, such as 1-fluoro-2,4-dinitro benzene [114] and *p*-toluenesulfonyl chloride [115] have been used for detection in UV-Vis, but with lower sensitivity.

Post-column procedures use derivatization with OPA-MERC, and fluorescence detection [116–119]. Most of the procedures utilize the commercial post-column reactor developed for the analysis of carbamates, but the procedure is slightly different; glyphosate is oxidized in glycine which is then coupled to OPA-MERC to give a fluorophor. The US EPA method consists of a direct injection of the aqueous samples, with a limit of detection of 6 $\mu\text{g/l}$ in drinking water. Other methods commonly reported combine an enrichment step in order to obtain lower LODs. The extraction of glyphosate from aqueous matrices is difficult owing to its high solubility in water and requires the use of anion exchangers. The separation of glyphosate and AMPA is easily performed using a cation-exchange column.

3.3.3.6.2. Diquat and paraquat. These widely applied bipyridinium-type salts are difficult to analyze by GC. Although hydrogenation has been performed to make them volatile [120], their analysis is usually performed by LC or, more recently, by CZE. The LC method is described in the US EPA Method 549.1. Diquat and paraquat are quaternary amines that can be separated easily using ion-exchange LC or ion-pairing LC, and are easily detectable by UV DAD. Because of their characteristic UV spectra they cannot be analyzed at the same wavelength; paraquat is detected at 257 nm diquat at 308 nm. Figure 3.5 shows that the detection limits obtained using a microbore C_{18} column (100×2.1 mm i.d.) operating in the ion-pairing mode can be in the low nanogram level [121]. With an off-line trace-enrichment step from 250 ml of water sample, a final extract in 250 μl , and an injection volume of 10 μl , the minimum detectable concentration is in the range 0.4–0.5 $\mu\text{g/l}$.

3.3.3.6.3. Aminotriazole (or amitrole). This pesticide is widely used for agriculture and industry. Its low volatility, high polarity, and high solubility in water (280 g/l) make it impossible to analyze by gas chromatography, and very difficult to extract from water, although GC analysis after acetylation has been reported. A review was recently published, summarizing all the possibilities for both its extraction and its detection after UV [122]. Aminotriazole is too polar to be analyzed by reversed-phase chromatography, where elution with pure water as the mobile phase occurs close to the void-volume retention time. Normal-phase chromatography is not the best technique, because of the impossibility of injecting a large amount of aqueous solvents on the columns used in the normal-phase mode and to the low solubility of aminotriazole in organic solvents. However, since it can be ionized at low pH, ion-pair chromatography or exchange chromatography are the appropriate methods. The detection by UV is not sensitive at all owing to the lack of chromophore, even when derivatization techniques are used. The most sensitive detection is electrochemical,

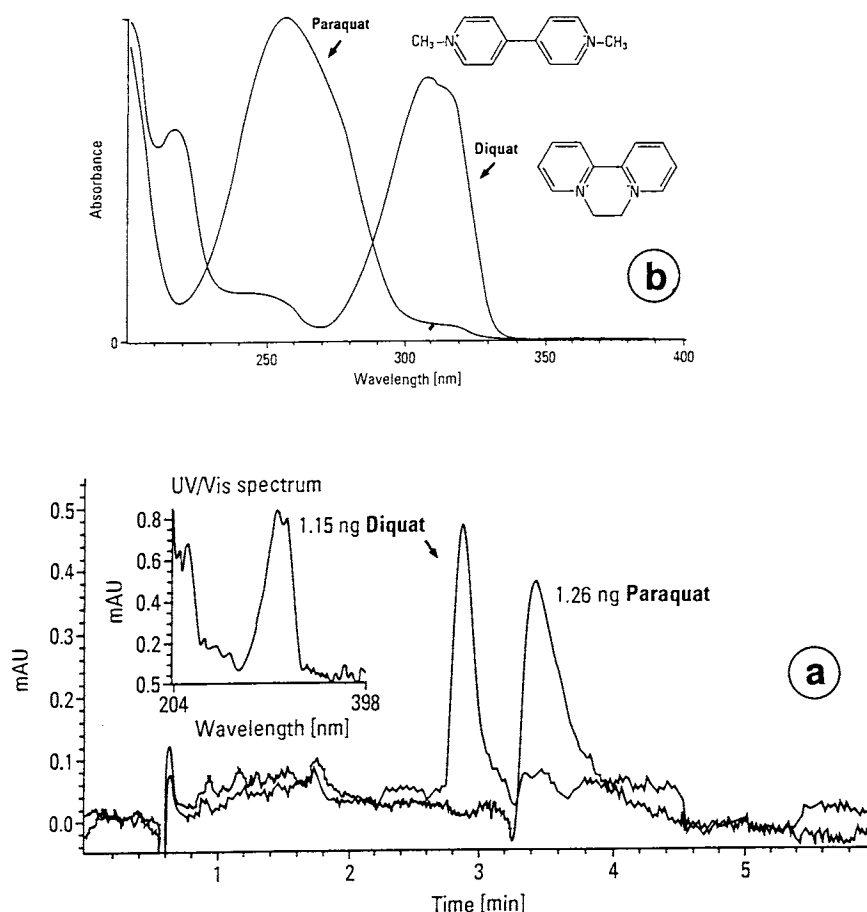


Fig 3.5. (a) Analysis at the nanogram level of diquat and paraquat and (b) UV spectra of diquat and paraquat. Column, 100 × 2.1 mm i.d. ODS Hypersil, 5 μ m; mobile phase, 0.1% hexane sulfonic acid and 0.35% triethylamine, pH 2.5 (H_3PO_4); flow rate, 0.4 ml/min; injection volume, 10 μ l; UV DAD.

and Fig. 3.6 shows the high sensitivity that can be reached; the chromatogram presented corresponds to the direct injection of 0.3 ng on a cation-exchange column. With the available concentration techniques, no robust methods can detect aminotriazole routinely below the 0.3–0.5 $\mu\text{g/l}$ level in drinking-water.

3.3.4. EPA methods

The EPA methods using LC are listed in Table 3.5. These complement the GC-methods reported in Table 3.3, to cover almost all the pesticides and transformation products on the NPS list. These methods include multiresidue analysis, as for chlo-

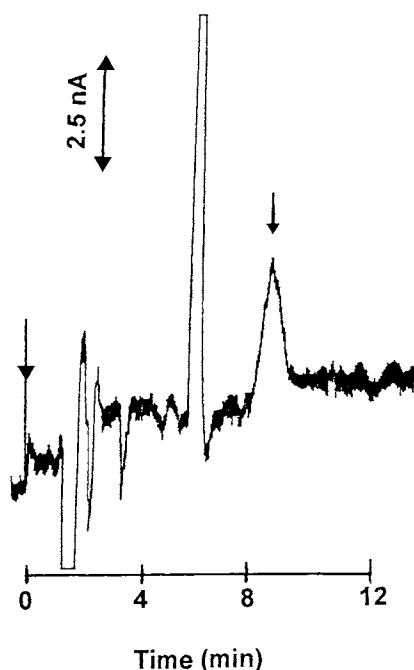


Fig. 3.6. Detection limit of aminotriazole obtained by direct injection of $100\ \mu\text{l}$ of water spiked with $3\ \mu\text{l}$ of aminotriazole ($0.3\ \text{ng}$ injected). Analytical column, cation-exchanger PRP-X200, $10\ \mu\text{m}$, $150 \times 4.6\ \text{mm}$; mobile phase, $\text{HNO}_3\ 2 \times 10^{-3}\ \text{M}$ with 30% methanol, v/v; flow rate, $1\ \text{ml/min}$; electrochemical detection at $0.75\ \text{V}$ (versus Ag/AgCl); from Ref. [122].

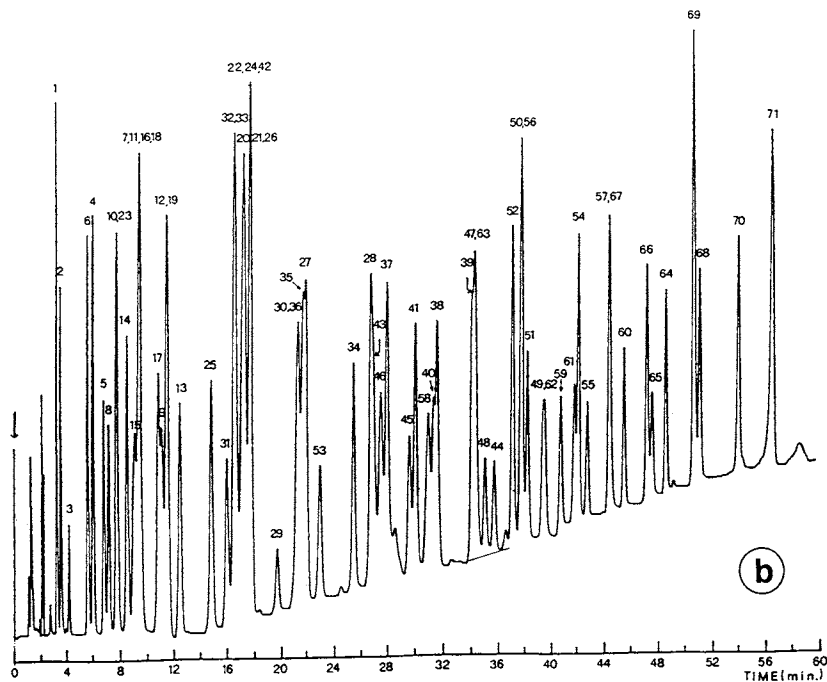
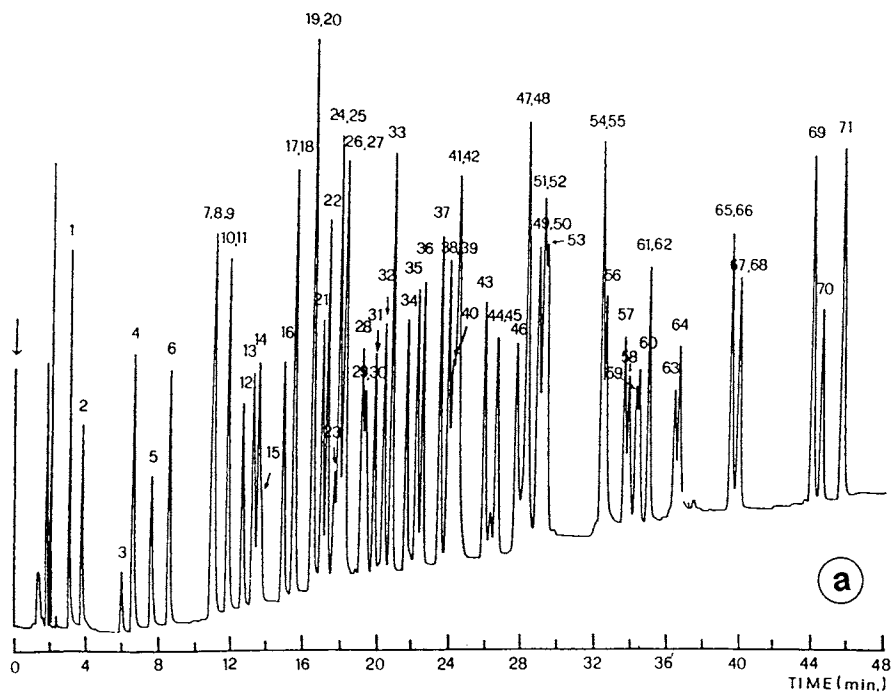
minated pesticides, or are specific for “unusual” pesticides, as for glyphosate, diquat and paraquat. Method 531, which uses post-column reactions with fluorescence detection, provides good detection limits from the direct injection of $200\ \mu\text{l}$ of aqueous samples, and shows thus the high sensitivity of this detection mode. In the last few years the first on-line SPE methods were introduced by the US EPA for the determination of chlorinated phenoxyacids in water matrices, as also indicated in Table 3.5. This represents progress in the US EPA methods and, in a way, a closer approach to European monitoring programmes, such as that in the Rhine basin where automated on-line SPE techniques are currently applied. More details on-line SPE techniques are given in Chapter 5.

3.3.5. Multiresidue methods

The potential of LC for multiresidue analysis was shown by Di Corcia and Marchetti [99]. In one run, they have achieved the separation of 71 neutral and basic pesticides using a $250 \times 4.6\ \text{mm}$ i.d. C_{18} column and a linear water–acetonitrile gradient. The chromatogram is shown in Fig. 3.7a,b; a separation of the same mixture is

TABLE 3.5
SUMMARY OF THE EPA-NPS METHODS USING LC TECHNIQUES

<i>EPA Method 531.1 (NPS Method 5):</i>	Determination of <i>N</i> -methylcarbamoxyl oximes and <i>N</i> -methylcarbamates in ground water by direct-aqueous-injection-LC, post-column derivatization and fluorescence detection
–	Direct injection of water samples
–	LC column: C ₁₈ silica column
–	After elution, hydrolysis with 0.05 M NaOH at 95°C, reaction with <i>o</i> -phthalaldehyde (OPA) and 2-mercaptoethanol to form highly fluorescent derivatives
–	Detection with fluorescence
–	Compounds detected (LOD in the range 0.5–4.0 µg/l): aldicarb, aldicarb sulfone, aldicarb sulfoxide, baygon, carbaryl, carbofuran, 3-hydroxycarbofuran, methiocarb, methomyl, oxamyl
<i>EPA Method 555:</i>	Determination of chlorinated acids in water by LC with UV DAD
–	Liquid-solid phase extraction (20–100 ml); esters are included after hydrolysis with 6 N NaOH followed by acidification with H ₃ PO ₄
–	LC column: C ₁₈ silica column, 250 × 4.6 mm i.d.; primary column: ODS-AQ, 5 µm (YMC Ltd) and confirmatory column; 300 × 4.6 mm i.d., Nova-Pak C ₁₈ (Waters)
–	Mobile phase: water (H ₃ PO ₄ , 0.025 M)–acetonitrile gradient from 10% to 90% acetonitrile in 30 min
–	UV DAD detection
–	Compounds detected (LOD in the range 0.1–0.5 µg/l with extraction of 100 ml): acifluoren, bentazone, chloramben, 2,4-D, 2,4-DB, dicamba, 3,5-dichlorobenzoic acid, dichlorprop, dinoseb, 5-hydroxydicamba, MCPA, MCPP, 4-nitrophenol, pentachlorophenol, picloram, 2,4,5-T, 2,4,5-TP
<i>EPA Method 547:</i>	Determination of glyphosate in drinking-water by direct-aqueous-injection-LC, postcolumn derivatization and fluorescence detection
–	Direct injection of filtered water samples (200 µl)
–	LC column: cation-exchange LC column, 250 × 4.6 mm, Bio-Rad Aminex A-9, K ⁺ form at 65°C; guard column: C ₁₈ packing
–	Mobile phase: isocratic, 0.005 KH ₂ PO ₄ and methanol, 96:4 v/v
–	After elution at 65°C, oxidation with calcium hypochlorite; the product (glycine) is coupled with <i>o</i> -phthalaldehyde (OPA) and 2-mercaptoethanol at 38°C to give a highly fluorescent derivative
–	Detection with fluorescence, excitation at 340 nm, and detection of emission measured at >455 nm
–	Detection limits: 6.0 µg/l in reagent water, 9.0 in ground water
<i>EPA Method 549.1:</i>	Determination of diquat and paraquat in drinking-water by liquid-solid extraction and LC with UV detection
–	Solid-phase extraction from 250 ml of sample, adjusted to pH 10.5 using a C ₈ cartridge or disk prepared for the reversed-phase, ion-pair mode
–	LC column: Hamilton PRP-1, 5 µm, 150 × 4.1 mm at 35.0°C
–	Mobile phase: isocratic, ion-pair mobile phase
–	UV detection at 308 nm (diquat) and 257 nm (paraquat) or UV DAD
–	Detection limits: 0.4 µg/l for diquat and 0.8 µg/l for paraquat



represented using a cyano propyl-modified silica column as the confirmatory column. One can see that the retention order is not exactly the same, because of the mixed retention mechanism involved with this type of column. The term multiresidue analysis can be properly applied to this separation, since the standard mixture contains pesticides used as insecticides, herbicides, fungicides, nematocides, and acaricides, representative of all the common groups, i.e., carbamates, phenylureas, triazines, triazinone, organophosphorus, acetanilides, dinitroanilines and even one organochlorine compound (peak 70) and one pyrethroid (peak 71). In the same paper, the separation of 18 varied acidic pesticides is also presented (see Fig. 3.8) using the same columns as for the base-neutral mixture, but with an acidic mobile phase.

Multi-residue analysis is valuable, provided all the compounds are extracted together in one run. Although it is not discussed in this chapter, it is worthy of mention that these compounds can be extracted together, with complete recoveries, using a 2 l sample and a 300 mg Carbo-pack cartridge (graphitized carbon black).

Many other examples of multiresidues containing a larger or smaller number of pesticides can be found in the literature. A multiresidue analysis of some phenylureas, triazines and acetanilides is the object of an official method (German DIN standard 38407 F12) and a representative example can be found in next chapter (Figs. 4.13 and 4.14), with LODs for each pesticide below the 0.1 µg/l level in drinking water.

On-line solid-phase extraction (SPE) and LC is particularly well adapted to multi-residue analysis. Relevant examples can be found in Chapter 5, and Fig. 3.4 presents

Fig. 3.7. Separation of a standard mixture containing 71 base-neutral pesticides using (a) a C₁₈ silica column and (b) a confirmatory cyanosilicone column. From Ref. [99] with permission. (a) Column, LC-18 DB, 250 × 4.6 mm i.d., 5 µm, Supelco; mobile phase, linear gradient from 80% 10⁻³ M phosphate buffer at pH 6.7 and 25% acetonitrile to 85% acetonitrile in 45 min; flow rate, 1.5 ml/min. (b) Column, LC-CN (cyano) 250 × 4.6 mm i.d., 5 µm, Supelco; mobile phase, initial composition 88% 10⁻³ M phosphate buffer at pH 6.7 and 12% acetonitrile, held for 7 min, and linear gradient to 57% acetonitrile after 50 min; flow rate, 1.5 ml/min. (a,b) Detection UV at 220 nm amount of each pesticide injected 10–150 ng. Peak numbers: (1) oxamyl; (2) methomyl; (3) mevinphos I; (4) chloridazon; (5) dimethoate; (6) mevinphos II; (7) hexazinone; (8) aldicarb; (9) metoxuron; (10) simazine; (11) bromacil; (12) monuron; (13) cyanazine; (14) metribuzin; (15) dichlorvos; (16) propoxur; (17) carbofuran; (18) pirimicarb; (19) atrazine; (20) chlortoluron; (21) fluometruon; (22) carbaryl; (23) diazinon; (24) isoproturon; (25) ethiofencarb; (26) monolinuron; (27) diuron; (28) difenoxuron; (29) metobromuron; (30) paraoxon; (31) propachlor; (32) propham; (33) propazine; (34) propanil; (35) terbutylazine; (36) dichlobenil; (37) linuron; (38) azinphos-methyl; (39) chloroxuron; (40) fenamiphos; (41) chlorbromuron; (42) molinate; (43) propyzamide; (44) parathion-methyl; (45) chlorpropham; (46) metolachlor; (47) procymidone; (48) malathion; (49) fenitrothion; (50) rotenone; (51) azinphos-ethyl; (52) neburon; (53) eptam; (54) fenitrothion; (55) parathion-ethyl; (56) sulfallate; (57) coumaphos; (58) cycloate; (59) phorate; (60) phoxim; (61) disulfoton; (62) pirimiphos-methyl; (63) butylate; (64) methoxychlor; (65) chlopyrifos; (66) pendimethalin; (67) pirimiphos-ethyl; (68) trifluralin; (69) bromophos-ethyl; (70) DDT; (71) fenvalerate.

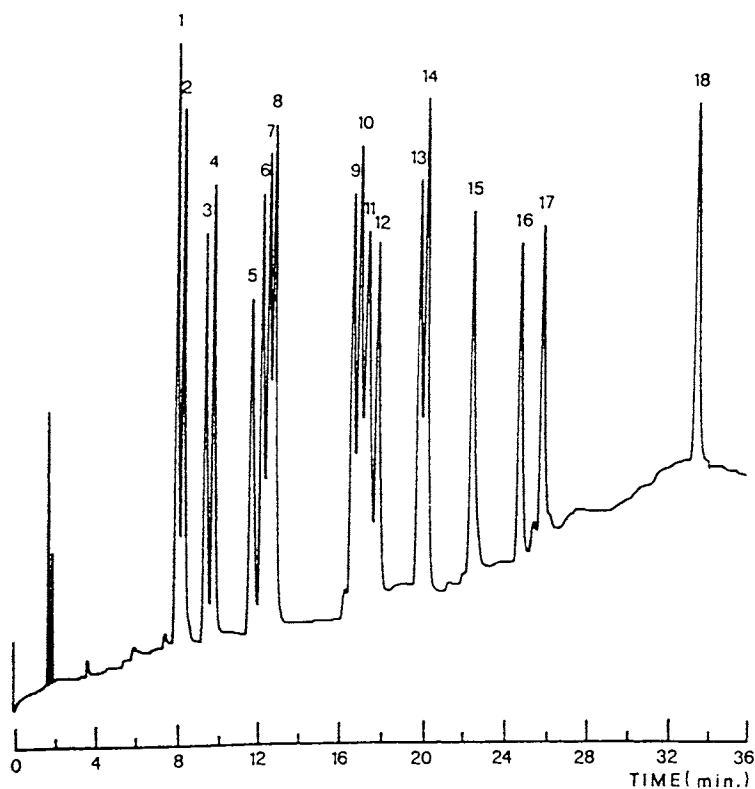


Fig. 3.8. Separation of a standard mixture containing 18 acidic pesticides. From Ref. [99] with permission. Column, LC-18 DB, 250×4.6 mm i.d., $5 \mu\text{m}$, Supelco; mobile phase, linear gradient from 50% acidified water with 0.12% TFA (v/v) and 50% of a premixed methanol-acetonitrile (82:18, v/v) to 78% premixed organic mixture in 34 min; flow rate, 1.5 ml/min. Detection UV at 230 nm amount of each pesticide injected 43–172 ng. Peak numbers: (1) bentazone; (2) dicamba; (3) bromoxynil; (4) dinitro-*o*-cresol; (5) coumafuryl; (6) 2,4-D; (7) ioxynil; (8) MCPA; (9) dichlorprop; (10) mecoprop; (11) warfarin; (12) 2,4,5-T; (13) 2,4-DB; (14) MCPB; (15) 2,4,5-TP; (16) dinoseb; (17) dinoterb; (18) pentachlorophenol.

a multiresidue analysis routinely performed in our own laboratory for the control of herbicides in ground water. From a 150 ml sample, LODs are below than $0.1 \mu\text{g/l}$ in drinking water and in the range $0.1\text{--}0.5 \mu\text{g/l}$ in surface water. Liska et al. have presented a rapid screening on-line SPE-LC method designed for an early-warning system of over 50 pesticides in surface water [123]. It allowed the separation of almost all the compounds with LODs of about $1\text{--}5 \mu\text{g/l}$ after a preconcentration of 30 ml of water.

Multiresidue methods have been developed using LC with post-column-reaction detection. Over 100 analytes from the EPA's National Survey of Pesticides in

Drinking Water Wells were screened for a response using post-column photolysis followed by fluorescence, electrochemical, or conductivity detection [124]. LC-photolysis, with electrochemical or fluorescence detection, was shown to be suitable and complementary. The first combination responds to several sulfur-containing pesticides whereas the of combination photolysis-FD responds to many nitrogenous pesticides. The conductivity detection was not suitable for sensitive multiresidue determination. Approximately half of the compounds from the list could be determined in low nanogram amounts using the two detection systems and multiseparation with gradient reversed-phase LC.

3.3.6. Further trends

It is clear that LC is complementary to GC for the analysis of pesticides and that the two methods are necessary in any environmental laboratory. Almost all the pesticides can be directly analyzed by LC, and one strong point of LC is its high suitability for multiresidue analysis and its capability for automation with the pre-treatment step. The most relevant point is that polar transformation products of pesticides can be analyzed much better by LC than by GC, so LC has its great advantage in the analysis of new and polar metabolites in water samples.

LC is now implemented in many new official methods. Most probably, LC will also involve the use of microcolumns, owing to their easier coupling with certain MS interfacing systems, and following the trends for solvent reduction. We can certainly expect LC to have an increasing impact on pesticide analysis in the near future.

3.4. THIN LAYER CHROMATOGRAPHY

In recent years, thin layer chromatography (TLC) has gained attention in pesticide residue analysis because it has evolved into an instrumental technique that offers automation, especially with the introduction of the automated multiple-development technique (AMD) by Burger and commercialized in the late 1980s by Camag (Switzerland) [125]. This technique can provide reproducible gradients and was used to screen water samples for pesticides over a wide range of polarity [126]. AMD TLC is described in the German Official Method for water analysis, DIN 38407, Part 11 [127].

3.4.1. General aspects of modern thin layer chromatography

Modern TLC is an instrumental technique, distinguished from conventional TLC in many ways, as recently described in an extensive review [128]. It involves precise control of the various steps, from the application of sample to the chromatographic development and recording of the chromatogram. Thanks to automation, the results are more reproducible and accurate.

As in LC, TLC separations are governed by the distribution of analytes between a stationary phase and a mobile phase, with different migration rates of the analytes along the stationary phase. However, the methods differ greatly in the format of the stationary phases, the elution mode and kinetics, and the efficiency.

3.4.1.1. Thin layer plates

The layers have been greatly improved and are now prepared with narrow size-distributions, mean particle diameters in the range 5–15 μm and thicknesses from 0.05 to 2.0 mm. Although plates prepared from various reversed-phase silicas are available, silica gel is the most widely used stationary phase in contrast to the situation with LC. One reason for the less numerous applications is that C_{18} reversed-phase layers cannot be developed with highly aqueous solvents, because the hydrophobic repulsive forces are stronger than the capillary forces which move the solvent through the layer unless force-flow development is applied [128]. The water-wettability is important and only layers with low carbon contents and particle size of 10–15 μm can be employed for reversed-phase TLC.

3.4.1.2. Force-flow development

In normal-phase TLC, migration of the mobile phase through the layer is governed by capillary forces. The velocity reduces as the distance between the solvent-front and the solvent-entry point increases; it is therefore not constant throughout the chromatogram. As capillary forces are not able to provide the optimum velocity required to reach the minimum plate height at any migration distance, force-flow development is applied. Force-flow is usually performed by using the “overpressure development chamber”, in which the layer is sealed by applying hydraulic pressure to a polymeric membrane in contact with its sorbent surface, and the mobile phase is forced through the layer by an external pump [129].

3.4.1.3. Single and/or multiple development

The separation of more than six to eight components with base-line resolution is always difficult in TLC separations employing a single development under capillary-controlled flow conditions. Because most separations are performed on silica gel, the optimization of a separation is obtained by varying the mobile-phase composition. Computer-assisted strategies have been described for this optimization [130]. In trace analysis, when large volumes of extracts containing the analytes are applied, band broadening cannot be avoided at the starting point in single development methods, and additional band broadening occurs throughout the chromatographic development. In order to reduce this band broadening, multiple development has been performed which allows an increase in the separation performance and the application of mobile-phase gradients.

The multiple development technique involves successive, repeated developments of the layer, with the removal of the mobile phase between developments. The plate

is developed rapidly over a fixed distance using the same mobile phase and between developments the mobile phase is removed and the plate dried. This method can be performed with incremented multiple development. In this case, the first development distance is the shortest and in each subsequent development either the distance or time is increased in order to obtain the desired resolution for the separation. The last distance is the longest one. One important advantage of this method is that it provides the refocusing effect, owing to the spot-preconcentration mechanism. At the start of each development the solvent front contacts the bottom edge of the chromatographic zone first, and this region of the zone starts moving forward towards the molecules which are still ahead of the solvent front. One disadvantage of the multi-step process is its resulting long separation time.

By changing the composition of the mobile phase for each successive cycle, a stepwise solvent gradient can be generated. Gradients of increasing solvent strength are used for the separation of complex mixtures by separating just a few compounds in each step. Individual components are usually identified and quantified by scanning the intermediate steps. Another method consist of separating the sample for the shortest distance in the strongest mobile phase and then, in each subsequent development, using mobile phases of reducing solvent strength. This strategy allows the final separation to be recorded as a single chromatogram and is easy to automate, as described below.

3.4.1.4. Automated multiple development (AMD)

AMD TLC is a relatively new technique. The design of the instrument enables one to perform chromatographic separations under well defined and controlled conditions with the result of providing reproducible retention data, as in GC and LC. Moreover, AMD TLC allows an inherent permanent refocusing of the analyte bands. It can provide a separation efficiency considerably higher than conventional TLC, with 20–30 baseline separations per AMD chromatogram, and reproducible gradient elution on silica gel plates [131].

The solvent is started with the strongest eluent, and its principal function is band focusing. It is applied only for a short distance and is usually carried out ten times. The selected starting eluent should provide weak retention for all the sample analytes so a narrow band is formed gathering all the analytes capable of migration; undesired matrix components are left on the injection spot. Then, the gradient proceeds in successive cycles to solvents of lower strength, with development over increasing migration distances, the last development being the longest. The term “universal gradient” describes a conventional normal-phase chromatography TLC gradient that starts with a very polar solvent and is varied through a moderately polar solvent to a non-polar solvent. The central solvent, or “basis” solvent, and to a certain extent the non polar solvent, determine the separation. In most cases, this solvent is dichloromethane or an ether.

The usual development length is 80–90 mm and the increments are generally be-

tween 1 and 5 mm. A typical complete program comprises 20–30 cycles. One unique feature of the gradient technique, which has no parallel in LC, is that abrupt changes of the developing solvents are possible. A separation can start with an alkaline solvent gradient and end with an acidic solvent mixture.

3.4.2. Application of AMD TLC to the screening of pesticides

We now describe the screening techniques according to the Official Method DIN 38407, part II.

Silica gel plates, 20 × 10 cm preferably with a 100 μ m layer thickness are used. Two gradient programs are proposed. In the first, the plates are developed using a 33-step gradient. The first ten steps are performed with the same solvent composition and the gradient starts, as shown in Fig. 3.9a. A typical separation of a mixture of 11 pesticides, representing a wide variety of chemical structures, is shown in Fig. 3.9b. This chromatogram constitutes nine individual plots of the reflectance at nine wavelengths between 190 and 350 nm. A reflectance spectrum can be obtained from each spot of the thin-layer plate at any time after drying.

The DIN method includes 22 plant-treatment agents determinable by this method using gradient I of Fig. 3.9a, or a second one, starting with a mixture containing methyl-*t*-butyl ether and 10% acetonitrile, and then with an increasing content of hexane. With these two gradients, the separation of the 22 compounds is presented. Each example of a chromatogram contains a maximum of eight peaks, so that identification is made by the UV spectrum and the retention time. The limitation of the method is the number of peaks that can be separated in one run, and the maximum number of base-line-resolved peaks seems to be closer to 20 than 30, as is illustrated by a recently published chromatogram showing the separation of 16 pesticides [132]. There is a large difference between this and the screening power of an LC column as seen by comparing the chromatogram in Fig. 3. 7. The suitability of the DIN method has been shown for 283 pesticides from different classes, and the data for migration distances, the UV spectra and the instrumental detection limits have been given [126]. Only 18 pesticides show detection limits of more than 100 ng applied, and can therefore not be analyzed from 1 l of drinking water samples without further treatment. The pesticides were extracted from 1 l of drinking water by means of LLE or LSE and screened with the gradient I. The confirmation of the suspected positive pesticide residues was performed using a second analysis, applying a special gradient optimized for the individual pesticide classes.

3.4.3. Further trends

AMD TLC can be used to screen for pesticide residues in drinking water samples and has been applied to drinking water extracts. The problem of interfering compounds is to be expected more with surface water samples. For multiresidue screen-

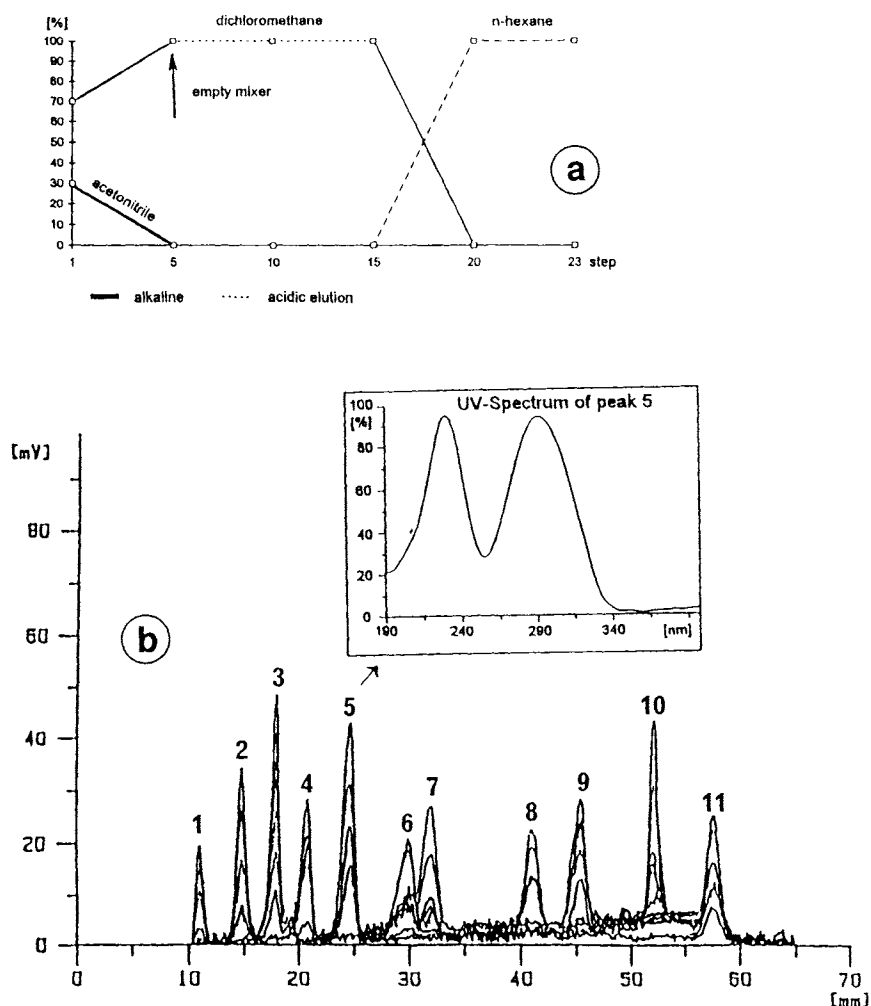


Fig. 3.9. (a) Elution gradient used for screening pesticides using the DIN 38407, part 11 procedure and (b) multi-wave length chromatogram of a mixture of 11 pesticides with UV spectrum of chloridazon. (a) Acidic solution obtained by addition of 0.1% formic acid and basic solution by addition of 0.1% of 25% ammonia in water. (b) Solutes: (1) clopyralid acid; (2) triclopyr acid; (3) bifenox; (4) atraton; (5) chloridazon; (6) sethoxydim; (7) atrazine; (8) iprodione; (9) desmedipham; (10) ethofumesate; (11) pendimethalin. From Ref. [126] with permission.

ing, the limitation of the technique is in its separation power. In real samples, overlapping of peaks with unknown compounds is expected, so identification via UV spectra is not straightforward and we can expect the full exploitation of the UV spectra and peak purity to take some time. In these conditions, the coupling with MS

is not easy. The use of another confirmatory method is necessary, so the technique as described is complementary to LC or GC [123].

At the moment, the screening technique using AMD TLC is far from being widely adopted in environmental laboratories. Several requirements should be considered for screening purposes. First, a good screening method would not involve sample preparation and/or should be very rapid and not expensive in comparison to LC. This is the case for screening methods such as immunoassays. These conditions are not yet met by AMD TLC, because the apparatus is not cheap, the method is not really rapid, and it requires sample preparation. Moreover, there is competition from LC screening. The same approach, i.e., collecting retention times and UV spectra for all the pesticides, with a universal gradient involving the separation of 15–20 peaks, can be very rapid and simple, within 10–15 min. This rapid screening LC approach has not been developed, certainly because much more information can be expected from a LC run. The future will tell whether chemists will adopt AMD TLC as a first-screening method.

3.5. CAPILLARY ELECTROPHORESIS

In recent years, capillary electrophoresis (CE) has developed into an exciting and extremely powerful technique. Initially, CE was applied mainly in biochemical analysis but more recently its applicability has been demonstrated in all fields of chemical analysis. As usually happens when a method develops rapidly, not all reported publications are relevant, and our aim is to discuss the potential of CE for monitoring pesticides in water. Because CE offers rapid and efficient separations of ionic, ionizable, and neutral compounds, and because it is based on a different separation mechanism, it is a valuable technique, and complementary to LC or GC.

3.5.1. General aspects of capillary electrophoresis

CE has become a powerful tool for the separation of a wide range of compounds, from small ions to large biological molecules such as proteins and oligonucleotides. The main separation modes of CE are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MEKC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF) and capillary isotachopheresis (CITP). This versatility of CE makes possible a wide range of applications. Some techniques, such as CGE and CIEF, are more specific of the separation of proteins or polysaccharides. Environmental analyses of herbicides are generally performed using CZE, MEKC or CEC [133].

In general, CE is a “nanoscale” separation technique, with a high resolving power and small sample requirement. It provides very reproducible and efficient separations. The capillary dimensions are $10\text{--}100 \times 2\text{--}200\text{ }\mu\text{m}$ i.d. The small volume of the columns limits the loadings onto the system to the range 1–60 nl. CE has attracted

attention because it permits the analysis of samples which are available in very minute amounts, such as some specific biological samples. That is not the case with pesticide samples, where there is no limitation on the sample, even after extraction. Environmental chemists are attracted by the technique's inherent very high efficiency, which is explained by the flat flow profile in the capillary and by the rapid separation. The column efficiency is about 10–20 times higher than that in LC. This high efficiency, combined with an optimized selectivity, allows the separation of a large number of solutes in a short retention time. Zone-broadening resulting from fittings and connectors can be avoided using on-column detection. The small dimensions of the capillary and on-column detection, allow detection in the femtomole range.

CZE is the simplest technique using buffer-filled capillaries for the separation of charged compounds. In fact, the principle of most of the capillary electrokinetic separation techniques lies between those of pure electrophoresis and chromatography. In MEKC, using the same experimental configuration as in CZE, charged compounds and neutral compounds can be separated according to their hydrophobicity, through partition between an aqueous electrolyte and a micellar non-polar pseudo-phase formed by the simple addition of an ionic surfactant. A capillary packed with a LC stationary phase is used in the CEC method, which is a complete hybrid between CE and LC.

CE and LC are often compared, and the complementarity of the techniques was illustrated with the detection of terbutylazine in ground water [134]. The different positions of the matrix interferences in the chromatogram and electropherogram next to the analytes of interest confirmed the complementarity of the information given by the two techniques.

Other advantages of CE techniques are their low-price of maintenance because of the negligible consumption of organic solvents and chemicals, and the low prices of the uncoated fused-silica capillaries, especially for the CZE and MEKC techniques.

3.5.1.1. *Capillary zone electrophoresis (CZE)*

The principle and the apparatus for CZE are very simple. An open tubular fused-silica capillary is filled with an electrolyte. The sample to be separated is injected at one end. Each end of the capillary is submerged in a buffer reservoir and an electric potential is applied. Migration of the charged analytes occurs in the sample, and their separation can be achieved because they have different migration velocities. The detection is at one end of the capillary.

The migration flow is the result of the electromigration and electro-osmosis. With a fused-silica capillary, electro-osmosis is the bulk-flow of liquid resulting from the effect of the electric field on counter ions adjacent to the negatively charged capillary wall. Because the wall of the fused-silica capillary is negatively charged under most pH conditions, there is a build-up of positive counter ions in the solution adjacent to the capillary wall. This layer of positive charge is drawn towards the negative electrode.

Joule heating, electro-osmosis, and zone dispersion are important factors to consider when performing any electrophoretic separation, because of the high electrical fields, the large surface-area-to-volume ratio, and the low viscosity of the supporting electrolyte of the CE separations. The proper control of these factors governs the success of the separation.

It is necessary to dissipate the Joule's heat that is generated as a result of the electric current passing through the electrophoresis buffer. This results in a temperature gradient which can induce remixing of the separated sample zones by natural convection, or by introducing a spatial dependence of the electrophoretic mobility [135]. In the late 1980s, the introduction of capillaries of diameter less than 200 μm resulted in a reduced production and better dissipation of heat generated by the Joule effect. The deleterious influence of convection phenomena has thus become almost negligible [136]. The separations can now be performed in free electrolytes, under even higher electric fields (50 kV/m). The presence of an electro-osmotic flow permits the convenient on-line detection of the solute, near to the same capillary's end.

The basic variables (analysis time, retention factors, contributions to plate height, resolution) are expressed in terms of the main operating parameters. Under optimized conditions (dispersion controlled by axial diffusion), the reported efficiencies are currently in the range 10^5 to 10^6 theoretical plates.

Interactions between analytes and the electrolyte components allow one to extend the range of application, for example by using complexation reactions. In addition, CZE has proved to be a powerful and flexible technique for the separation of stereoisomers. An interesting application deals with the CZE separation of phenoxy acid herbicides and related impurities, including positional isomers, originating from the production process [137]. Chiral separations of phenoxy propionic acid herbicides were achieved by adding a suitable cyclodextrin-type chiral selector to the electrophoresis buffer.

3.5.1.2. *Micellar electrokinetic capillary chromatography (MEKC)*

The principal limitation of CZE used to be its inability to separate neutral compounds. In 1984, Terabe and co-workers [138] introduced a modified version of CZE in which surfactant-formed micelles are included in the running buffer to provide a two-phase chromatographic system for separating the neutral compounds. The buffer is electro-osmotically transported through the capillary and the micelles are transported through the capillary by a combination of electro-osmotic flow and micelle electrophoretic migration.

The MEKC technique offers several advantages. Using the same instrumentation as CZE, MEKC is more versatile, since the electrophoretic mobility can be exploited for separating ionic species. Differences in phase distribution, more or less apparent to ion-polar chromatography, can be exploited for separating neutral compounds. Thus, MEKC provides some of the versatility of reversed-phase ion-pair chromatography along with the high efficiency of CZE.

3.5.1.3. Capillary electrochromatography (CEC)

CEC is a hybrid separation technique combining the stationary phase of liquid chromatography with the electrically driven mobile-phase transport of capillary electrophoresis. Unlike hydraulically driven LC, the movement of the mobile phase through a packed CEC column is not subject to back pressure, so there is the possibility of using small-particle packings with the resulting of a gain in efficiency. In many examples, capillaries packed with $3\text{ }\mu\text{m}$ particles of C_{18} silica are used. The application of the electric field (typical voltages of 10–25 kV) to the packed capillary and to the mobile phase generates the osmotic flow. By forcing the electrically driven liquid through the C_{18} silica capillary the analytes are separated by LC partition between the liquid and the stationary phase. For neutral analytes, the selectivity is identical in LC and in CEC, whereas it is different for ionic compounds because ions migrate at different rates in response to the applied electric field.

The high resolution in CEC is caused by the flat, plug like profile of the electro-osmotic flow, which results in a very sharp peak. Pumped systems, such as those used in capillary LC, give rise to a parabolic flow profile which gives a much broader minimum peak width. The routine application of CEC was recently demonstrated by incorporating a $75\text{ }\mu\text{m}$ i.d. capillary packed with $3\text{ }\mu\text{m}$ C_{18} silica and testing the reproducibility of separation of neutral compounds for a hundreds runs. The analytes were separated with an average of 180 000 plates/m in less than 8 min, and the retention factors were reproducible to better than 2% [139].

The advantage of CEC over CE is that neutral compounds can be separated together with ionic and ionizable analytes. CEC is more attractive than MEKC, because micelles lack selectivity, can cause high background effects on detection, present problems at high concentration because of the high current, and cannot be interfaced to a mass spectrometer.

The mobile phase selected is typically an aqueous buffer with sufficient conductivity to support the electro-osmotic flow. Usually, this is provided by selecting a pH in the alkaline range to ensure a consistent concentration in the solid-phase packing of residual silanol groups, which are responsible for generating the electro-osmotic flow.

3.5.1.4. Detection methods

The usual detectors are the same as those used in LC. With minor modifications, several of these detectors have been adapted to accommodate the use of the fused-silica capillary as the detector flow cell. Therefore, no extra fittings are required. However, many of these detectors suffer some loss in sensitivity owing to the reduced detection cell dimensions and the reduced volumes of CE. As for other methods, the performance criteria of detectors relate to their sensitivity, selectivity, linear range, and the overall system noise.

The most common detector for pesticides analysis is based on UV absorbance. High-quality fused silica capillaries should be used, since fused silica has a cut-off at

approximately 170 nm. Several methods are used in the instrumentation to increase the path length of the detection cell. Indirect detection is frequently used for the detection of inorganic ions. The optical systems are identical to those used for direct absorbance; the only difference in the indirect detection mode is that the electrophoretic buffer contains a UV chromophore.

Fluorescence detection is often used, especially in biological applications for analytes containing primary amines, such as amino acids, peptides and proteins. Post-column derivatization is more difficult because a sufficient sample volume, i.e., a few μl , should be available for derivatization. Despite this difficulty, a post-column reactor has been developed for detection, using OPA as derivatizing reagent.

The coupling with MS is certainly interesting, since MS is more easily coupled to small diameter columns. This is an increasing area especially with the introduction of the electrospray interface. One constraint lies in the selection of the aqueous buffers, as for LC-MS work.

3.5.1.5. *On-line trace-enrichment methods*

Despite the impressive CZE separations which have been demonstrated, it was concluded in 1990 that CZE was not sensitive enough for environmental analysis unless special injection tricks became available [140]. It is true that the current UV detectors were able to detect as little as 600 fg, so they competed with the most sensitive GC detectors. However, when the injection volume of several nanolitres is taken into account, the sensitivity expressed in sample-concentrations term was very poor, in the range of several hundred ppb or $\mu\text{g/l}$.

In practice, off-line extraction and concentration are being used to increase the overall concentration sensitivity. In addition, special injection methods have been developed for CZE which allow the introduction of samples as large as the entire volume of the separation capillary [141,142].

Nielen [140] has examined the various methods for sensitivity improvement in CZE and has distinguished several approaches. The first simply consists of improving the detection technologies, based on laser-induced fluorescence, amperometry, mass spectrometry or UV absorbance with extended path lengths. The second is to perform solid-phase extraction inside the capillary, by incorporating a short plug of a reversed-type of packing material at the beginning of the capillary, or by bonding a hydrophobic phase to the inner wall of the capillary. However, there is a risk of plugging the system with real samples, so one has to introduce samples which have been at least partially cleaned, and do not contain a percentage of organic solvent.

Another approach uses the on-line coupling of isotachopheresis (ITP) and CZE, which can allow the injection of volumes up to several tens of microlitres. ITP is an electrophoretic technique which is carried out in a discontinuous buffer system. The analytes are injected between a so-called leading (i.e., higher mobility) and a terminating (lower mobility) electrolyte. When the current has been switched on, a steady-state migration configuration will be established, in which the analytes migrate as

consecutive zones which are not diluted in the background electrolytes as in CZE. This makes on-line coupling of ITP and CZE attractive, and has been demonstrated by several authors. Using this technique, a sample volume as large as 90 μl could be accommodated, and the herbicides paraquat and diquat could be analyzed with a detection limit of 10^{-9} mol/l [143].

A promising approach for on-line trace-enrichment is the so-called field-amplified injection. This technique is based on the fact that the electrophoretic velocity of an ion depends linearly on the field strength, i.e., on the applied voltage divided by the length of the capillary. When an analyte is dissolved in a sample matrix which has a lower conductivity than the background electrolyte or buffer in the capillary, the analyte will experience a local increased field strength and will migrate with a higher velocity. When the analyte reaches the boundary between the sample-matrix zone and the buffer, it will slow down and stack into a zone much shorter than the original sample zone. Thus, it has been preconcentrated or focused on-column. The original sample can be injected either hydrodynamically or electrokinetically. The enrichment factor thus obtained could be further improved by pumping out the matrix zone using the electro-osmotic flow under reversed polarity conditions [141].

The performance and applicability of field-amplified injection was studied using the separation of the phenoxy acid herbicides, MCPP, 2,4-DP, MCPA, and 2,4-D. [140]. The optimized separation conditions permitted the separation of a 20 mg/l solution. The simple field-amplified sample allow to enrich of a factor ten, whereas a solution of 20 $\mu\text{g/l}$ could be analyzed directly by the injection of a large volume (35% of the entire capillary volume) using field amplification with matrix removal under reversed-polarity conditions. Sample pre-treatment was applied, but then minimized by using C_{18} membrane disks for simultaneous filtering and solid-phase extraction. Desorption from the disks directly into CZE sample-vials could be carried out using acetonitrile–buffer mixtures, thus providing a sample matrix having a sufficiently low and constant conductivity. It was possible to analyze the phenoxy acids at the 0.5 $\mu\text{g/l}$ level in river water from a sample volume of 40 ml.

3.5.2. Applications to the main groups of pesticides

CE techniques are complementary to LC and GC in the analysis of polar acidic and basic pesticides, since these compounds are not amenable to GC without derivatization, and their determination by LC may be of insufficient efficiency. From the literature we have selected some applications that are summarized below.

3.5.2.1. Triazines

The separation of triazines and their degradation products has been achieved using with CZE [144], and the quantitative determination of chlorotriazine in tap water was performed by MEKC after concentration using SPE [145]. Because *S*-triazines and their degradation products exist as ionic species at certain pH values, they are

good candidates for separation by CZE. The effects of buffer pH on the electrophoretic mobility of chloro-, hydroxy-, methoxy-, and thiomethyl-S-triazines have been studied in relation to their chemical properties [146]. Good separations were achieved for each class, in the form of their cations, except for chlorotriazines which have too low pK_a values (below 2). The separation of hydroxyatrazine metabolites, as their anions, could also be achieved at a high pH of around 8. The detection level calculated for most of the compounds was 0.05 mg/l of analyte in the injection solution.

CE in its free-solution mode has been used by the same authors [147] as a rapid tool for the quantification of hydroxyatrazine formed during the photodegradation of atrazine under a nitrogen and oxygen atmosphere in the presence or absence of humic substances in aquatic media. Hydroxyatrazine was found to be the main photodegradation product. The CE mode involved in this study was found to be very effective for the separation of the cationic hydroxytriazines from the anionic humic polyelectrolytes, using an analysis without a clean-up procedure.

3.5.2.2. *Phenoxyalkanoic acids and other acidic pesticides*

This group has received much attention because of the easy ionization of analytes at the pH of natural waters and also because some of the compounds, such as dichlorprop, are sold and applied as racemic mixtures. The separation by CE of several plant-growth regulators, including 2,4-D and 2,4,5-T, has been described, with the addition of cyclodextrins and cholic acid to the buffer in order to enhance the selectivity [148]. As already mentioned, CE was developed to separate the enantiomers [137]. A recent study used CZE modified with an appropriate chiral cyclodextrin for showing the enantiomeric selectivity in the environmental degradation of dichlorprop [149]. A similar CE method was developed to separate seven chlorophenoxy acids and their enantiomers. The cavity size and the concentration of cyclodextrins influenced the migration times of individual herbicides, and the seven compounds could be separated within 7 min, with two pairs of enantiomers being completely resolved. The dynamic range was 1–100 mg/l. By combining the method with a preconcentration using C_{18} disks, the herbicide detection limits in the environmental waters were below 1 $\mu\text{g/l}$ [150].

3.5.2.3. *Phenylureas*

The separation of the neutral phenylureas is usually achieved using MEKC. A rapid single separation of five phenylureas and four phenoxyalkyl acids was achieved by MEKC after different aliphatic alcohols were added to a phosphate buffer containing 0.05 M sodium dodecyl sulfate as surfactant [151]. The addition of an alcohol-chain improved the resolution of the ionic compounds. The effect of the chain length was studied and the results explained in terms of the variation of the aqueous polarity, the micelle surface-charge density, and the micellar volume.

Six urea herbicides were analyzed by MEKC using trialkyltrimethylammonium chloride and bromide surfactants. The separation was optimized by using decyl- or dodecyltrimethyl-ammonium chloride as micellar phase [152]. An efficiency of 150 000 plates/m was obtained and the separation was obtained within 15 min.

3.5.2.4. *Sulfonylureas*

In contrast to phenylureas, the sulfonylureas are ionizable and are well amenable to CE. The use of CZE with UV detection for the determination of metsulfuron and chlorsulfuron in tap water at the $\mu\text{g/l}$ level was described [153]. Water samples were spiked at the 0.1–5 $\mu\text{g/l}$ level and extracted using SPE prior to CZE analysis. The two herbicides were separated in 4 min, with detection limits of 0.1 $\mu\text{g/l}$ for both compounds. In another study by the same authors, the separation and detection of the metabolites of nine sulfonylureas in aqueous media was evaluated [154]. There was evidence of a relationship between the structures of the tested compounds and the metabolites formed. The same method was applied to the determination of chlorsulfuron, chlorimuron, and metsulfuron in soils [155].

3.5.2.5. *Highly polar pesticides (quats, glyphosate, aminotriazole, etc.)*

The cationic quaternary ammonium herbicides, diquat and paraquat, have also received some attention. CZE was used with acetonitrile (10%, v/v) in a phosphate buffer (100 mM, pH 4.0) using an optimum applied voltage of 12 kV. The method permitted direct analysis of water samples spiked at 0.1–0.5 ppm, with final confirmation also, since diode-array detection was used [156]. The detection limits were found to be in the range 15–20 fmol, with an efficiency of 200 000–300 000 theoretical plates/m. In another study, electrolyte running-solutions with different alkaline salts were investigated in an attempt to reduce adsorption of the solutes on the capillary wall [157]. The resolution of diquat and paraquat was found to be critical, and required well-established conditions of pH, temperature, buffer cation, and injection mode. The performance of the method was compared with reversed-phase liquid chromatography [158]. The detection limits, as defined by a signal-to-noise ratio of 3, ranged from 2.9 to 5.5 $\mu\text{g/l}$, and were similar for both techniques when the standards were dissolved in water. However, with real samples the response of CE was affected more by the nature of the sample matrix.

A method was developed for the analysis of glyphosate and aminomethylphosphonic acid (AMPA) in water using CE with indirect detection [159]. Tetradecyltrimethylammonium bromide was added as electroosmotic flow modifier in a 10 mM phthalate electrolyte. The analytes were separated in less than 4 min under reverse polarity conditions and detected indirectly. Response was shown to be linear in the range 1.7–170 mg/l glyphosate and 1.1–110 mg/l AMPA. With stacking from water, the limit of detection for glyphosate was 0.8 mg/l for a 6.7 nl injection. Field-amplified sample injection has enabled 2 $\mu\text{g/l}$ to be detected.

3.5.3. Multiresidue analysis

In a recent publication, LC and MEKC have been compared for the determination of a variety of pesticides mixtures [160]. These mixtures included triazine, phenyl-ureas, phenoxyalkyl acids, carbamates, and organophosphorus pesticides, selected because of their use in different combinations in commercial formulations. The best results have been obtained by using mobile-phase gradients in LC and by working with *n*-alcohols as mobile-phase-modifiers in MEKC. By observing the number of theoretical plates, *N*, in each method, it could be seen that although the value of *N* for the anionic compounds in MEKC was between 300 and 400 times higher than for LC, the difference was not so important for the neutral pesticides. This was explained by the fact that the addition of micelles to the CE system increased the band-broadening in the separation column. Neither method was sensitive enough for environmental analysis. SPE and special field-amplification injection were studied to enhance the sensitivity of MEKC methods. Field amplification injection was shown to be a rapid and inexpensive method, but is only applicable to the preconcentration of ionic compounds in the MEKC system and has a lower enrichment factor than SPE.

CE with UV detection and laser-induced fluorescence detection (LIF) has been used for analyzing a group of pesticides that has widespread use in the USA [161]. CE-UV was employed for the detection of atrazine, simazine, metolachlor, alachlor, dicamba, 2,4-D and chlorimuron-ethyl. Dicamba, 2,4-D, and chlorimuron ethyl were detected by CE-LIF following derivatization with 4-bromomethyl-7-methoxycoumarin for the first two compounds and with dansyl chloride following hydrolysis for the last one. After extraction from 1-l samples using Empore C₁₈ disks. The detection limits in fortified pond-water samples with CE-UV were 0.1 µg/l for the two triazines and metolachlor, and 1 µg/l for alachlor, although it was estimated at 10 ng/l for the compounds when detected by fluorescence. As mentioned earlier, the on-line coupling of sample pre-treatment using isotachopheresis and CZE was reported above as detecting these compounds with a detection limit of 10⁻⁹ mol/l [143].

3.5.4. Further trends

The literature contains many examples of pesticide residue analysis using CE. Obviously, CE, like LC, can be an appropriate method for multiresidue analysis but its best applications lie in the analysis of ionic/ionizable pesticides. Multiresidue analyses, as performed with LC methods and involving a large number of compounds, have not been obtained. The separations achieved so far have involved compounds having rather closely related physicochemical properties. CE is a technique complementary to LC and GC, and very helpful for polar ionized pesticides, which may provide some more analytical information. It is clear that CE is not intended to compete or to be used as an alternative technique. It has been used for studies on the

environmental behaviour of pesticides, to study degradation, and to identify metabolites.

It is likely that CE will become as widely accepted a technique as LC. However, this can only be a result of the actual attractiveness of this new, versatile technique for researchers, which explains the high number of applications found in the recent literature. One must be aware of its major drawback, the low loadability of the CE system, and there is still a need for developing highly sensitive and precise detection modes for environmental studies. Recently, the use of new cells that increase the optical path length up to 1–2 mm has allowed an enhancement of the sensitivity under CE-UV of one order of magnitude. In this respect, the newly developed system should permit more applications to trace analysis.

3.6. MASS SPECTROMETRIC METHODS

Mass spectrometry (MS) is recognized as a highly sensitive and specific technique that can be used for environmental organic analysis. The combination of chromatography and mass spectrometry (MS) gives the high discriminating power of one instrument with the high separating power of another so that complementarity of the qualification and quantitation performance of the individual techniques can be achieved. Thus, gas chromatography (GC) is an excellent quantitative but poor qualitative technique, and is very well matched with MS, which offers good qualification and poor quantitation. In GC-MS the selectivity is improved compared to MS alone, by the physical separation of the components of a mixture by chromatography prior to mass analysis. The selectivity can be enhanced either by the use of different reagent gases in positive and/or negative chemical ionization (PCI and NCI, respectively) or by the use of two or more analytical techniques in tandem, as in GC-MS-MS.

3.6.1. GC-MS using electron impact (EI)

Review articles [162–164] and books [165–167] have been published which describe fundamental aspects and applications of GC-MS to pesticide analysis together with tables of spectroscopic information. GC-MS can be applied in various ways to pesticide analysis: with electron impact (EI), positive chemical ionization (PCI), negative chemical ionization (NCI), and tandem MS (MS-MS).

GC-MS with EI is the MS technique most widely used by laboratories involved in environmental organic analysis. The most common practice is to identify the compounds of interest by library search and/or by performing a second injection with co-elution using authentic standards. The use of available libraries of more than 120 000 spectra for the identification of the different environmental compounds is of great interest [166]. A very useful book with the EI spectra of environmental priority pollutants has also been published [165]. Calibration of the instruments is carried out by

automatic programs such as the so-called AUTOTUNE program and using perfluorotributylamine. The US Environmental Protection Agency (EPA) has established some criteria for performing good calibrations of the instruments; these are defined in both EPA methods using GC-MS [168,169] and are indicated in Table 3.6.

GC-MS with EI usually gives good sensitivity and reproducibility of the spectral data can reveal structural information. A disadvantage of EI is that it does not always provide molecular weight information, as the molecular ion species, $[M]^+$ of many compounds is too transient to be observed in the mass spectrum. The reproducibility of EI data on a given mass spectrometer, and between different mass spectrometers using similar conditions, is generally good, so that rapid identification of spectra is possible by comparison with mass spectral databases. This technique is usually performed using three different types of instruments: quadrupole analyzers, ion traps and magnetic sectors. Depending on the instrument used, differences in relative abundances of the EI spectrum for a given compound have been observed. Thus, for organophosphorus pesticides it has been noticed that the use of a quadrupole analyzer may result in a lower abundance of the ions at high mass as compared to that from magnetic sector instrument, with differences varying from 3 versus 100% [164]. Similarly, when using an ion trap, which has a design completely different

TABLE 3.6
ION ABUNDANCE CRITERIA FOR BIS (PERFLUOPHENYL)PHENYLPHOSPHINE
(DECAFLUOROTRIPHENYLPHOSPHINE, DTPP): US EPA METHOD 525.1

Mass (<i>m/z</i>)	Relative abundance criteria	Purpose checkpoint
51	10–80% of the base peak	Low mass sensitivity
68	<2% of mass 69	Low mass resolution
70	<2% of mass 69	Low mass resolution
127	10–80% of the base peak	Low-mid mass sensitivity
197	<2% of mass 198	Mid-mass resolution
198	base peak or >50% of 442	Mid-mass resolution and sensitivity
199	5–9% of mass 198	Mid-mass resolution and isotope ratio
275	10–60% of base peak	Mid-high mass sensitivity
365	>1% of base peak	Baseline threshold
441	Present and <mass 443	High mass resolution
442	Base peak of >50% of 198	High-mass resolution and sensitivity
443	15–24% of mass 442	High-mass resolution, isotope ratio

All ions are used primarily to check the mass measuring accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The three resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test. To correct setting of the baseline threshold, as indicated by the presence of the low intensity ions, is the next most important part of the performance test. Finally, the ion-abundance ranges are designed to encourage some standardization to fragmentation patterns.

from a quadrupole analyzer and which claims equal or better sensitivity, remarkable differences in the EI spectra of chlorotriazines were noticed. For example, cyanazine showed a base peak at m/z 68 using an ion trap whereas with a quadrupole analyzer the base peak corresponded to m/z 225, with differences in the relative abundances in the two instruments of more than 50% [170,171]. The technique is adequate for environmental organic analysis since the limits of detection (LOD) are usually at the low ppb level for the determination of organic pollutants in water and soil samples, with absolute injected amounts below 1 ng, when working under full-scan conditions and with a scan range from m/z 30–50 up to 800. This scan range is common in most of the quadrupole and ion trap instruments, also called “bench tops”. When higher m/z values are needed, the use of quadrupole instruments up to m/z 3000 or magnetic sector instruments are required.

The sensitivity of GC-MS (and also of the other chromatography-MS combinations) can be increased by one or two orders of magnitude by using only a few selected ions, by the technique known as selected ion monitoring (SIM). The quantitation under GC-MS is usually achieved by using SIM, or by employing labelled compounds. A limitation of GC-MS is that the compounds should be sufficiently volatile to be GC-amenable, and so derivatization is needed for polar compounds.

3.6.1.1. EPA methods

The most common EPA method for determining pesticides in drinking water matrices involving GC-MS was described a few years ago [168]. It is a general method that provides procedures for the determination of organic compounds in finished drinking water, raw source water, or drinking water in any treatment stage. The method is applicable to a wide range of organic compounds that are efficiently partitioned from the water sample onto a C_{18} organic phase, in either cartridge or disk format, and are sufficiently volatile and thermally stable for GC determination. The method involves the preconcentration of 1 l of the water sample. The organic compounds are eluted from the cartridge or the disk with a small amount of dichloromethane, and concentrated further by evaporation of some of the solvent. An aliquot of the compounds is injected into a capillary GC-MS system. Compounds eluting from the GC column are identified by comparing their measured mass spectra and retention times with the reference spectra and retention times in a data base. The concentration of each pesticide is measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by a compound used as an internal standard. As internal standards, either acenaphthene-D10, phenanthrene-D10, or chrysene-D12 are used. The GC columns used are common to the EPA methods previously described in Section 3.2.5. The concentration-calibration range of the method is between 0.1 and 10 ppb, and the LODs and quantitation ions for a selection of pesticides are indicated in Table 3.7.

A new method for the analysis of pesticides and other organic chemicals in water samples was published a few years ago [169]. It corresponds to the US EPA method

TABLE 3.7
QUANTITATION IONS AND LIMITS OF DETECTION FOR A SELECTION OF PESTICIDES INCLUDED IN THE US EPA METHOD 525.1, AFTER EXTRACTION OF 1 l OF WATER USING EXTRACTION DISKS OR CARTRIDGES AND DETERMINATION BY GC-MS WITH AN ION TRAP MASS SPECTROMETER

Compound	Quantitation ion (<i>m/z</i>)	LOD (ppb)
Alachlor	160	0.092
Aldrin	66	0.083
Atrazine	200/215	0.140
Chlordane- α	375	0.384
Chlordane- γ	375	0.200
Chlordane (<i>trans</i> -nonachlor)	409	0.574
Endrin	81	0.160
Heptachlor	100/160	0.144
Heptachlor epoxide	81/353	0.244
Hexachlorobenzene	284/286	0.111
Lindane	181/183	0.041
Metoxychlor	227	0.084
Pentachlorophenol	266	47.648
Simazine	201	0.118

8270C. This method is particularly interesting for pesticide analysis since it includes a wide range of pesticides. Basically, the method used the same SPE procedure as the method described previously for drinking water, using 1 l of sample. The main ions used for quantitation and the LODs of the method are indicated in Table 3.8. This GC-MS method, using an appropriate sample preparation method, can also be used for solid waste matrices, soils, and air matrices. The estimated quantitation limits (EQLs) varied between 10 and 100 ppb. Such limits are much poorer than those for drinking water (see Table 3.7) as a result of the more complex matrix.

3.6.1.2. Environmental applications

Derivatization techniques have been reported for a variety of thermally labile pesticides, and are followed by GC-MS. Stan and Klaffenbach [33,38] reported the derivatization of urea using heptafluorobutyric anhydride, and of carbamate pesticides using acetic anhydride. Other authors also used heptafluorobutyric anhydride for various urea herbicides and combined the derivatization technique after SPE for their in water samples. The detection limits were of the order of 10–50 ng/l using 1 l water samples [172]. Carbamate pesticides were also determined by GC-MS after on-line derivatization using flash-heater methylation with trimethylsulfonium hydroxide. In this case also, the method was combined with SPE. The preconcentration of 1 l of water allowed LODs to be achieved in the range of 25–50 ng/l [173]. Acidic herbicides have been determined traditionally by GC-MS after derivatization. Various official methods of analysis, from the UK [4] and other published methods [174,175]

TABLE 3.8

QUANTITATION IONS OF PESTICIDES INCLUDED IN THE US EPA METHOD 8270C, AFTER EXTRACTION OF 1 l OF WATER USING EXTRACTION DISKS OR CARTRIDGES AND GC-MS DETERMINATION AFTER SOLID PHASE EXTRACTION OF 1 l OF WATER: ESTIMATED QUANTITATION LIMITS VARIED BETWEEN 10–100 ppb (PESTICIDES ARE LISTED BY ELUTION ORDER FROM THE GC COLUMN)

Compound	Quantitation ion (<i>m/z</i>)
Dichlorvos	109, 185, 79, 145
Mevinphos	127, 192, 109, 67, 164
Naled	109, 145, 147, 301, 79, 189
Diallate (trans/cis)	86, 234, 43, 70
Demeton-O	88, 89, 60, 61, 115, 171
Dicrotopos	127, 67, 72, 109, 193, 237
Trifluralin	306, 43, 264, 41, 290
Bromoxynil	277, 279, 88, 275, 168
Monocrotopos	127, 192, 67, 97, 109
Phorate	75, 121, 97, 93, 260
Sulfallate	188, 88, 72, 60, 44
Demeton-S	88, 60, 81, 89, 114, 115
Dimethoate	87, 93, 125, 143, 229
Carbofuran	164, 149, 131, 122
Dioxathion	97, 125, 270, 153
Terbufos	231, 57, 97, 153, 103
Dinoseb	211, 163, 147, 117, 240
Disulfoton	88, 97, 89, 142, 186
Fluchloralin	306, 63, 326, 328, 264, 65
Mexacarbate	165, 150, 134, 164, 222
Phosphamidon	127, 264, 72, 109, 138
Methyl parathion	109, 125, 263, 79, 93
Carbaryl	144, 115, 116, 201
Malathion	173, 125, 127, 93, 158
Kepone	272, 274, 237, 178, 143, 270
Fenthion	278, 125, 109, 169, 153
Parathion	109, 97, 291, 139, 155
Carbophenthion	157, 97, 121, 342, 159, 199
Isodrin	193, 66, 195, 263, 265, 147
Captan	79, 149, 77, 119, 117
Chlorfenvinphos	267, 269, 323, 325, 295
Crotoxyphos	127, 105, 193, 166
Phosmet	160, 77, 93, 317, 76
EPN	157, 169, 185, 141, 323
Tetrachlorvinphos	109, 329, 331, 79, 333
Fensulfothion	293, 97, 308, 125, 292
Ethion	231, 97, 153, 125, 121
Captafol	79, 77, 80, 107
Dinocap	69, 41, 39
Methoxychlor	227, 228, 152, 114, 274, 212

Phosalone	182, 184, 367, 121, 379
Azinphos-methyl	160, 132, 93, 104, 105
Leptophos	171, 377, 375, 77, 155, 379
Mirex	272, 237, 274, 270, 239, 235
Coumaphos	362, 226, 210, 364, 97, 109
Aldrin	66, 263, 220
Lindane	183, 181, 109
4,4'-DDD	235, 237, 165
4,4'-DDE	246, 248, 176
4,4'-DDT	235, 237, 165
Dieldrin	79, 263, 279
Endosulfan I	195, 339, 341
Endosulfan II	337, 339, 341
Endosulfan sulfate	272, 387, 422
Endrin	263, 82, 81
Endrin aldehyde	67, 345, 250
Endrin ketone	317, 67, 319
Heptachlor	100, 272, 274
Heptachlor epoxide	353, 355, 351

use trifluoranilide or pentafluorobenzyllic derivatization. Other GC methods for pesticides such as paraquat and diquat involve a prior dehydrogenation step [120].

The presence of organophosphorus pesticides [4,19,171,176–179], triazines [60, 170,171,180–182], fungicides [183] and triazole pesticides has been reported. Multiresidue methods for rapid screening of pesticides in water matrices usually involve the preconcentration of 1–2 l of water samples, and the use of SPE and GC-MS analysis. The limits of detection are at the US EPA level [168,169] or lower, i.e., at the low ng/l level.

Depending on the pesticide type, different diagnostic ions are monitored. Chlorotriazines are characterized by a base-peak that usually corresponds to $[M - CH_3]^+$ or $[M]^+$, with other diagnostic ions formed by the loss of $[C_2H_5NH]^+$ or $[C_3H_7NH]^+$ [170,171]. Characteristic ions indicating the functional-group structure of the different organophosphorus pesticides are assigned to $[(CH_3O)_2PO]^+$, from, e.g., fenitrothion, $[(CH_3O)_2PS]^+$ from e.g., fenchlorphos, or $[(HO)_2PS]^+$, e.g., from parathion-ethyl, with other main ions corresponding to losses of OH, C_2H_4 , ClC_2H_4 , and ClC_4H_8 and Cl, depending on the compound. The molecular weight fragment is also obtained for most of the organophosphorus pesticides, with relative abundance of 25–100%. A typical GC-MS chromatogram obtained after preconcentration of an estuarine water sample enriched onto a C_{18} disk, for the determination of fenitrothion and various transformation products in the Ebro delta area is shown in Fig. 3.10.

The coupling of solid-phase micro-extraction (SPME) followed by GC-MS has also been used for the determination of various pesticides in water matrices [184]. The SPME GC-MS method was applied to 45 nitrogen- and phosphorus-containing

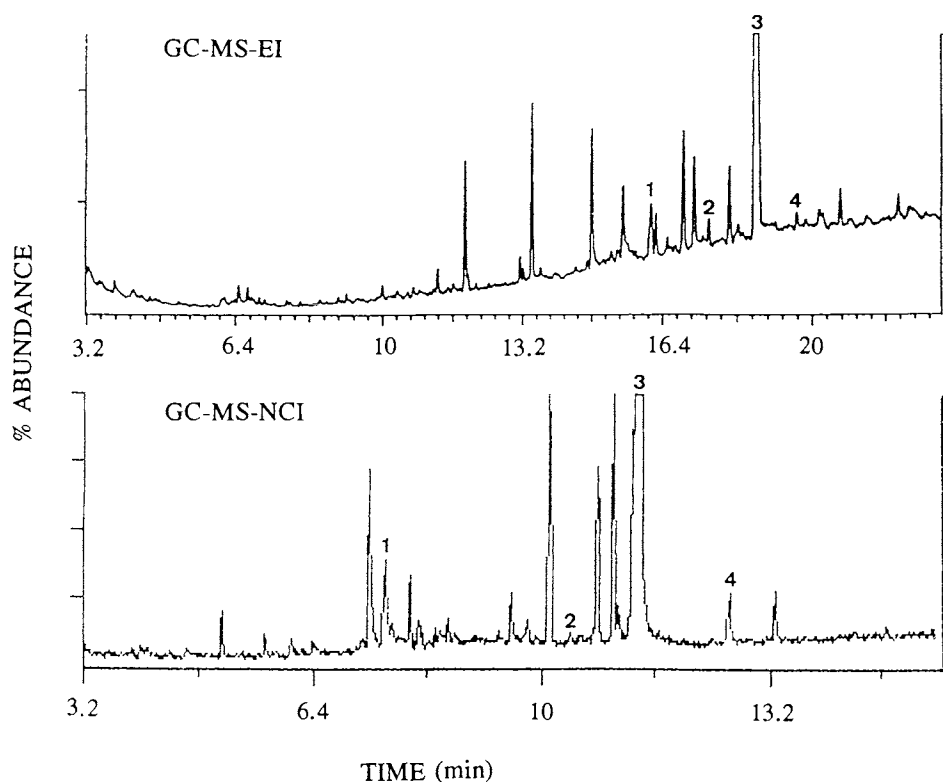


Fig. 3.10. Total ion current GC-MS chromatogram of an extract of estuarine-water samples of the Ebro delta (Tarragona, Spain) obtained after enrichment of 1 l of water onto C_{18} disks. (A) GC-MS with EI using a DB-1701 column and (B) GC-MS with NCI using a DB-5 column. Compounds identified were: (1) 3-methyl-4-nitrophenol, (2) fenitrooxon, (3) fenitrothion and (4) *S*-methyl isomer of fenitrothion.

pesticides of the US EPA Method 507. Analytes were extracted into a bonded polydimethylsiloxane phase coated on a fused silica fibre and then thermally desorbed into the GC injector and analyzed. Thirty-nine pesticides were measured at levels varying from 0.01–1 ppb, much lower than the US EPA method. The authors showed examples of stream water samples from the US. By using SPME GC-MS it was feasible to identify atrazine and alachlor at levels of 0.03 ppb, whereas the current US EPA method did not detect these two analytes since its LODs are of the level of 0.1–0.2 ppb. Corn herbicides have received much attention in the monitoring programmes in the USA and Europe, and GC-MS methods are being increasingly used. In this respect, the GC-MS investigation of many triazine metabolites has been performed by using an existing derivatization procedure [185]. A method for the low concentrations of acetochlor, a herbicide which is being increasingly used in corn cultivation in addition to the most common herbicides such as alachlor and atrazine,

has been published [186]. It involves the use of water samples of 1 l and reaches LODs of 1.5 ppt by using selected-ion monitoring.

Although most of the work on the determination of pesticides in water and other environmental samples has been performed using GC-MS with EI, quadrupole, or ion-trap instruments, a few papers have reported the use of GC-high resolution GC-MS for the determination of triazine pesticides in water [187]. Levels of atrazine below parts per trillion could be determined in water by combining the technique with a preconcentration on SPE cartridges. Dide-alkylatrazine was also determined in water samples by GC-high resolution MS after using graphitized carbon black. This material was needed for the SPE since the compound has a lower breakthrough volume than atrazine, but in this way it could be determined at the 2 ppt level [187]. GC-Ion-trap MS is also being increasingly used for the monitoring of various pesticides in environmental samples, following the release of new instrumentation and the capabilities of MS-MS [188–190].

Photolysis is one of the main non-biotic degradation processes that affects the transformation of pesticides in the aquatic environment. The coupled GC-MS with EI has been employed for characterization of the photolysis products of alachlor, and has also been reported to offer many possibilities for the identification of various transformation products [191,192]. Figure 3.11 shows the GC-MS EI spectra of various metabolites obtained after the photocatalyzed treatment of alachlor on TiO_2 and with a preconcentration using SPE disks. Many of the metabolites of alachlor formed under laboratory conditions were identified in ground water samples in the USA [193]. This indicates that the photo-oxidation experiments reported under laboratory conditions can be extrapolated to the field, as regards the pesticide metabolites formed. The use of GC-MS is essential; the identification of unknown metabolites is impossible otherwise.

The development of on-line coupling of liquid chromatography (LC) with GC-MS has been applied for the determination of triazine herbicides in surface and drinking waters [194,195]. The advantages of this new method are that not much sample manipulation is needed and that LOD in the low ppb range are obtained. With only 50 ml of water, atrazine could be analyzed at the 10 ppt level in drinking water samples.

3.6.1.3. *Further trends*

The use of GC-MS with EI is certainly the most widely employed technique in environmental pesticide analysis for confirmation and quantitation purposes. Although it is simple, reliable, and fast, emphasis should be placed on certain aspects: (i) the use of ion traps, quadrupoles or magnetic sectors in many cases gives differences in the relative abundances of the compounds analyzed and so difficulties in comparing spectra from different instruments, (ii) the compounds should be sufficiently volatile or derivatized prior to GC-MS injection, which is a disadvantage for the more polar and involatile environmental samples, (iii) significant clean-up is re-

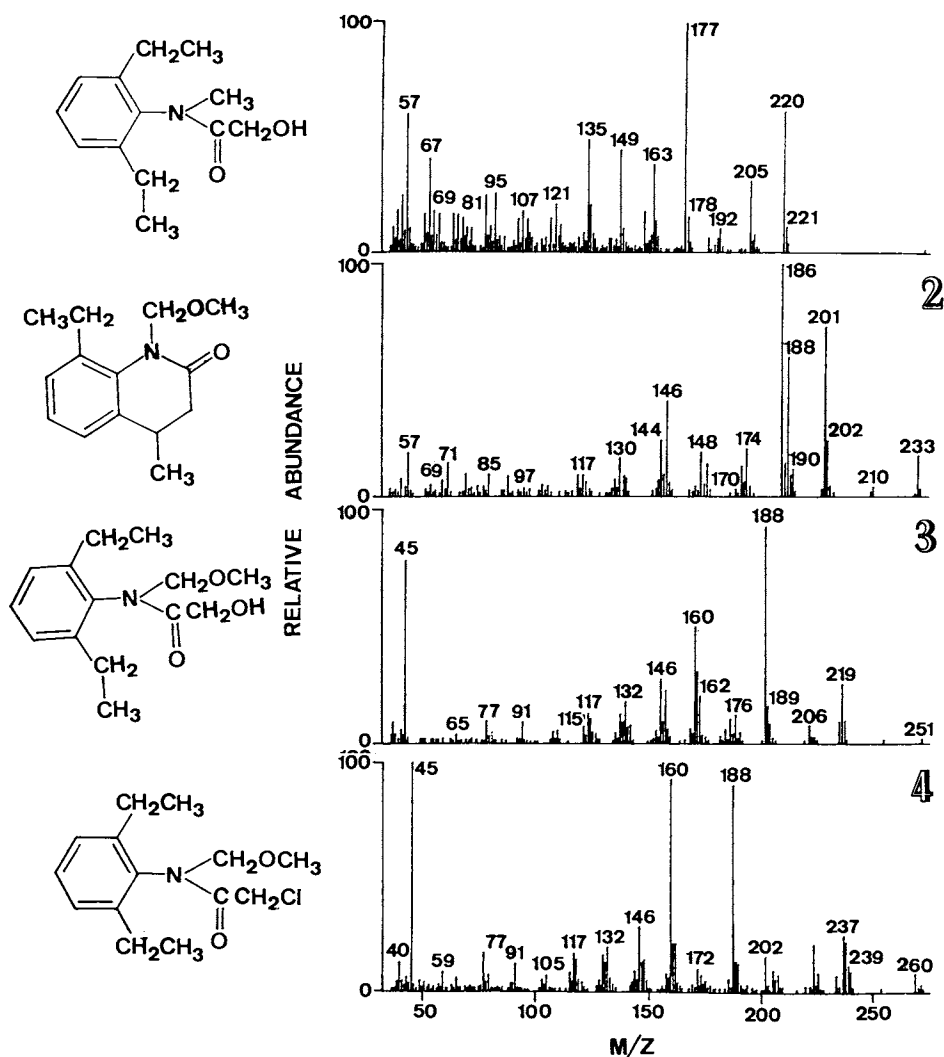


Fig. 3.11. MS spectra obtained under GC-MS EI conditions of alachlor (compound 4) and various metabolites: (1) 2-hydroxy-2,6-diethyl-*N*-methylacetanilide, (2) 8-ethyl-1-methoxymethyl-4-methyl-2-oxo-1,2,3,4-tetraquinoline, and (3) hydroxyalachlor. These compounds were identified in an extract of the photocatalysis of alachlor with TiO_2 and the solution was preconcentrated onto C_{18} Empore disks and injected onto GC-MS.

quired for dirty environmental samples prior to the GC-MS injection, otherwise column and source contamination will lead to problems in analytical performance. In the past few years a tendency has been observed towards the use of GC-MS with SIM for quantitative purposes, instead of its only being a qualitative confirmation technique for use after another GC detector such as ECD or NPD. The new

generation of ion trap detectors is also currently being used for pesticide monitoring, and there is an increasing use of GC-MS with EI for this purpose. All these facts can be explained by the reduced price of GC-MS instrumentation together with an increased availability of instrumentation in the different laboratories involved in pesticide control.

3.6.2. GC-MS with chemical ionization

GC-MS with either positive and/or negative chemical ionization (PCI and NCI, respectively) is currently employed in environmental organic analysis. Chemical ionization is considered a soft ionization technique that employs a chemical ionization reagent gas, generally methane but it can be isobutane or ammonia, at a source pressure between 0.1 and 2 Torr. The optimum reagent gas pressure used is strictly dependent on the reagent gas used [196,197]. The source temperature is another important parameter, which affects the fragmentation patterns and sensitivity. From the papers published in the literature we should mention an excellent treatise on the fundamental aspects of chemical ionization mass spectrometry and the research carried out on the environmental organic analysis of priority pollutants, mainly using NCI [197]. Of the two modes of operation, PCI has been employed widely for obtaining molecular weight information that conventional EI spectra do not give. Combined EI-PCI sources have been available for many years. For many compounds, the sensitivity of PCI is similar to that of EI, e.g., for chlorotriazines [171]. The different ions obtained in PCI depend on the compound and reagent gas employed. In this regard a typical spectrum, using methane as reagent gas, has as base peak the $[M + H]^+$ ion, and other peaks corresponding to $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$ together with some ions diagnostic of the molecule. With ammonia, the base peak can be either $[M + H]^+$ or $[M + NH_4]^+$, depending respectively on whether the proton affinity of the compound is higher or lower than that of ammonia.

3.6.2.1. Environmental applications

Methane was used as reagent gas in the determination of a variety of compounds such as chlorotriazine herbicides [171,198,199], organophosphorus [19,171,177,200,201] or carbamate insecticides [202] and acidic herbicides, after derivatization [4,203]. Other reagent gases employed were ammonia [199] for chlorotriazines and ammonia and isobutane for carbamate insecticides [202,204]. Although, in principle, a group of compounds of the same type will give a better response under one of the operational modes of chemical ionization, it can happen that some particular compounds, with remarkable structural differences, give a better signal under the other chemical ionization mode. Such is the case for organophosphorus pesticides that generally give better signals under NCI, but for some exceptions, e.g., crufomate, fenamiphos, fenthion, methamidophos, sulfotep and triazophos, PCI was recommended [201].

The increasing use of GC-MS with NCI in environmental organic analysis is particularly a result of the fact that most of the compounds of environmental interest contain electron-withdrawing groups, e.g., PCBs, nitro-PAHs, or organophosphorus pesticides having nitro- or chloroaromatic groups. A stabilization of the negative charge by electron capture is then feasible, leading to a better signal under the negative-ion mode. Ion-forming reactions under NCI are of different types, such as resonance- and dissociative-resonance-electron capture, ion-molecule reactions, radical-molecule reactions, wall-neutral interactions and ionization-neutralization-reionization reactions. It is clear that the formation of negative ions under electron-capture negative-ionization conditions is influenced by the presence of reagent gas impurities, the charge density, the composition of the plasma, the long ion-source residence times, and the elevated pressure. The technique is very useful in environmental analysis since it provides the two main features desired: selectivity and sensitivity. A disadvantage is that no libraries of spectra are available and the spectra obtained are very much instrument-dependent. One of the major problems encountered in GC-NCI-MS is in comparing the spectra obtained using different instruments. The LOD for a series of compounds of environmental interest were highly variable between instruments, which was attributed mainly to the "effective electron temperature", caused by the different focusing and extraction fields of each instrument [204].

The application of GC-MS with NCI to pesticide analysis has been reported for the determination of organophosphorus pesticides, and especially for compounds of the parathion group. The formation of the $[M]^-$ ion as base peak for the parathion group of compounds is a consequence of the aromatic ring structure of these organophosphorus pesticides which is easily stabilized under negative-ion conditions by the nitro group. Losses of Cl, such as CH_3Cl and HCl have been observed for chlorine-containing organophosphorus pesticides. The base peaks have corresponded either to the different functional group fragments or to the thiophenolate anions formed by transfer of the aromatic moiety from the oxygen to the sulfur atom, which was observed for phosphorothionate pesticides containing an aromatic moiety with electronegative groups [19,171,177]. Figure 3.10 shows the traces from an extract of an estuarine water containing fenitrothion and various metabolites, which has been analyzed by GC-MS with EI and NCI.

The development of scan-function and methane gas control systems has permitted the production of classical chemical ionization spectra in GC-MS with an ion trap, thus allowing direct comparison with reference spectra developed on magnetic and quadrupole instruments. Applications of GC-ion trap MS using chemical ionization conditions were reported by the group of Cairns, for a variety of pesticides, demonstrating comparability with conventional GC-MS conditions [189].

Further research has been carried out to increase selectivity by using other reagent gases instead of methane. In this case, the mass spectrum pattern can show important changes, with an enhancement of selectivity, thus providing a useful way for the characterization of pollutants. Methylene chloride at a pressure of 1 Torr was used as

reagent gas for performing chloride-attachment NCI MS for a variety of organophosphorus pesticides, in a similar way to that previously reported for organochlorinated compounds. In general, the functional group of the organophosphates was the base peak, with values for the attachment $[M + Cl]^-$ ion which varied, depending on the compound, from 1 to 100%. This chloride attachment ion is very useful in pesticide screening applications since it could allow an unequivocal identification of the pesticide [163]. Methane-enhancement, with argon as reagent gas, was used for the characterization of chlorotriazines, giving the formation of $[M + 14]^-$ and $[M + 28]^-$ adducts which were due to $[M + CH_2]^-$ and $[M + C_2H_4]^-$, respectively [205]. Interesting peaks were obtained by methane-enhanced NCI using oxygen for the characterization of a variety of organophosphorus pesticides which led to ion molecule reactions and the obtention of, for example $[M - HCl + O_2]^-$ [201].

3.6.2.2. Future trends

We note that GC-MS, mainly with NCI, is an appropriate technique for the confirmation of a great variety of environmental pollutants, and has the major advantage of selectivity that can be enhanced further by using various reagent gas mixtures. The increasing use of this technique is confirmed by the fact that in the last few years many of the GC-MS manufacturers have included PCI and NCI as options in their bench top instruments. In addition, chemical ionization techniques usually provide molecular-weight information on the analytes which, in many cases, EI spectra do not.

However, there are some deficiencies. They are: (i) there is no possibility of inter-comparison of spectral data between laboratories, and each laboratory needs to build up its own libraries, with the additional cost of obtention of standards; (ii) the ion source much more easily becomes “dirty”, because of the use of a high pressure gas operating, in many cases at a low source temperature such as 150°C, (iii) the analysis of environmental samples requires the use of extensive clean-up methods prior to the GC-MS with NCI analysis. As mentioned in (ii), the ion-source is more easily “dirtied” than in the case of EI operation, so the samples injected need to be as clean as possible.

3.6.3. GC-MS-MS

The different techniques and applications of MS-MS have been reported in a book by Busch et al. [206]. In general, a MS-MS consists of an ion source, two mass analyzers separated by a fragmentation region, and an ion detector. Such an arrangement can be used to separate individual components in a mixture or to obtain additional structural information on a single component. After mass selection of the characteristic ion of the analyte by the first mass analyzer, this parent ion undergoes collisionally activated dissociation (CAD) through collisions with neutral gas molecules (helium, argon or xenon) in the fragmentation region to yield various daughter ions.

The four most common MS-MS operating modes of tandem MS are: daughter scan, parent scan, neutral loss scan and multi-reaction monitoring (MRM). Up to six or more tandem MS options are possible, such as MS-MS-MS, but two of them are the most commonly used instruments: the first is the “hybrid MS”, which consists of a double-focusing instrument with electrostatic field (E), magnetic (B) configuration, which is followed by a collision quadrupole and finally by a mass analyzer quadrupole (= EBQQ). The second is the triple stage quadrupole, which consists of three quadrupole filters. The “hybrid MS” can be used either as a high-resolution MS or as MS-MS whereas the triple stage quadrupole can only be used as a MS-MS instrument.

3.6.3.1. Environmental applications

Although CAD MS-MS has scarcely been applied to environmental analysis, the development of “hybrid MS” instruments has permitted the accurate determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in environmental samples at the ppt level by using the MRM mode of specific daughter ions or by using high resolution. However, it was stated that GC-high resolution MS surpasses GC-MS-MS in its detection limits, linearity, and reproducibility [163].

Note that most work reported using GC-MS-MS involves the use of triple quadrupole MS. This is mainly a result of its lower price compared to a hybrid instrument and also to its much easier operation. The daughter scan mode is particularly useful for environmental organic analysis since, in this mode of operation, a parent ion characteristic of the analyte is selected in the first mass analyzer, it is fragmented by CAD in the fragmentation region, and then scanned in the second mass analyzer to obtain a daughter mass spectrum. By analogy to a normal mass spectrum, the daughter mass spectrum can be used for identification of an analyte by standard mass spectral interpretation or by matching the spectrum to an authentic sample. Such a system is currently applied to confirm a variety of pesticides, such as chlorotriazine herbicides [207,208], and organophosphorus and carbamate insecticides [163]. An example of daughter ion mass spectra is given for atrazine in Fig. 3.12, where the atrazine daughter ions spectra obtained for the parent $[M]^+$ and $[M - CH_3]^+$ ions are shown. First, it should be pointed out that the CAD spectrum for atrazine under the two different parent ions, $[M]^+$ and $[M - CH_3]^+$, is completely different. When $[M]^+$ is used as the parent ion, the daughter ion formed resembles the fragmentation obtained by conventional GC-MS in the EI mode [170,171]. Thus, CH_3 , C_3H_6 and $[C_3H_7NH]^+$ losses are observed. The daughter ions obtained when the $[M - CH_3]^+$ ion is used as the parent ion correspond to ring-opening reactions, resulting in signals at m/z 132, 104 and 96, thus indicating the presence of a C_2H_5 group. The ion at m/z 158 corresponds to a loss of C_3H_6 and CH_3 groups, while the fragment ion at m/z 71 confirms the presence of the C_2H_5 group and a secondary amine structure. Such additional structural information is very useful when confirmation of chlorotriazines in environmental matrices is needed. In addition, by monitoring the transition corre-

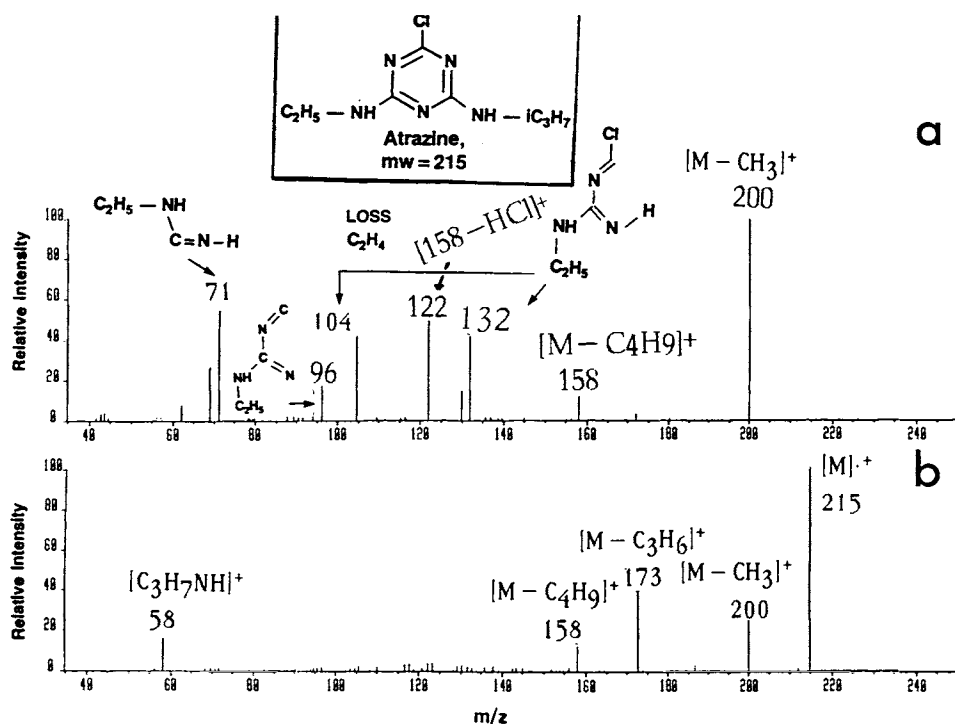


Fig. 3.12. Atrazine daughter ion spectra obtained in GC-MS-MS spectra of atrazine using collisionally induced dissociation of (a) $[M - CH_3]^+$ and (b) $[M]^+$ ions.

sponding to the loss of methyl, an increase in selectivity can be achieved, thus making the method suitable for environmental analysis [208].

3.6.3.2. Future trends

In summary, GC-tandem MS offers many possibilities in environmental organic analysis. It has two main advantages, in giving additional structural information compared to conventional GC-MS, and selectivity, by monitoring specific transitions, with minimum sample clean-up, thus allowing the use, if necessary, of direct MS-MS confirmation. The disadvantages of such a technique are: its cost, which is greater for conventional GC-MS, and difficulties in use, which are also much greater than in GC-MS and can lead to problems in reproducibility of spectra. In the last few years, a few ion trap instruments have the option of performing MS-MS spectra and it seems that this mode of operation will be used increasingly for the unequivocal confirmation of pesticides in environmental samples [189,190]. The price of a GC-ion-trap MS-MS is very attractive compared to a conventional triple quadrupole instrument, so its introduction into research and routine environmental laboratories is increasingly taking place.

3.6.4. LC-MS

The on-line combination of liquid chromatography (LC) and mass spectrometry (MS) provides a very convenient and robust technique for the analysis of polar pollutants. Five books and book chapters [167,209–215] have been published in the last few years, providing excellent overviews of the fundamental aspects and environmental applications of LC-MS.

The main problem to overcome in LC-MS is the transfer of analyte from the liquid phase into a high-vacuum gaseous phase. To accomplish the analyte transfer, different interfacing techniques have been developed over the past decade. The commercially available interfaces that are often applied can be divided into two major groups: the nebulization interfaces and the analyte-enrichment interfaces [214]. In the former, the eluent is nebulized by means of heat (thermospray, TSP), or pressure, or by the application of an electrical field (electrospray, ESP) at the tip of the spray capillary. Also, combinations of nebulization techniques have been developed (pneumatically assisted electrospray, and the heated pneumatic nebulizer). Within this group we give examples of pesticide analysis using four different types of interfaces: TSP, ESP, high-flow pneumatically assisted electrospray (ion spray, ISP), and atmospheric-pressure chemical ionization (APCI).

In the analyte enrichment interfaces, the analyte is separated from the solvent flow after nebulization, using a beam separator (particle beam, PB), or the subsequent evaporation of the eluent in different vacuum regions (moving belt). An excellent review on the use of the particle beam (PB) has been published in the last few years [216].

In this chapter, we report on the application of LC-MS to environmental pesticide analysis using the various interfaces which are commercially available. First, the current US EPA methods using LC-MS are discussed.

3.6.4.1. EPA methods

Of the different nebulizer types of interface methodologies, the TSP and PB interfacing systems have been widely used in environmental analysis. The US EPA has published two methods involving PB [217,218] and one involving TSP [219]. Recently, the comparison between the US EPA method 515.1 for chlorinated pesticides in water, involving derivatization and GC-ECD determinations, and the use of LC-PB-MS, have been carried out [220].

Method 553 involves the extraction of 1 l of water by dichloromethane LLE or LSE, followed by LC-PB-MS [217]. The pesticides determined include carbaryl, diuron, linuron, rotenone and siduron, with LODs varying between 4 and 31 ppb. The ion abundance criteria for calibration of the instrument are given in Table 3.9. Similarly, US EPA Method 8325 also uses PB-MS and is applicable to the same list of compounds. In this case the method is applied to waste water matrices, but the criteria reported in Table 3.9 are also valid. This method is just an extension of the

TABLE 3.9

ION ABUNDANCE CRITERIA FOR BIS(PERFLUOPHENYL)PHENYL PHOSPHINE (DECAFLUOROTRIPHENYLPHOSPHINE, DTPP): US EPA METHOD 8325 INVOLVING LC-PB-MS

Mass (<i>m/z</i>)	Relative abundance criteria	Purpose checkpoint
77	Present, major ion	Low mass sensitivity
168	Present, major ion,	Mid-mass sensitivity
169	4–10% of 168	Mid-mass resolution and isotope ratio
271	Present, major ion	Base peak
365	5–10% of base peak	Baseline threshold check
438	Present	Important high mass fragment
458	Present	Molecular ion
459	15–24% of mass 458	High mass resolution and isotope ratio

All ions are used primarily to check the mass measuring accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The three resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test. to correct setting of the baseline threshold, as indicated by the presence of the low intensity ions, is the next most important part of the performance test. Finally, the ion abundance ranges are designed to encourage some standardization to fragmentation patterns

previous method to more complex matrices. In this method it is indicated that the LODs are compound and matrix dependent. This is known in environmental analysis, and specially using LC-MS techniques. In complex waste water matrices the LODs necessarily will be poor as compared to clean drinking-water matrices. LC-PB-MS has been implemented by the EPA specially for the possibility of identifying unknown analytes in complex waste waters using the power of EI spectra.

The comparison of US EPA method 515.1 and LC-PB-MS for the determination of various acidic chlorinated acids in water has recently been reported [220]. In this case, 11 water samples were extracted with SPE using graphitized carbon black, followed by LC-PB-MS using SIM. The LODs for a variety of chlorinated acids varied from 0.7 up to 7 ppb when LC-PB-MS was used whereas with GC-ECD (with a prior derivatization with diazomethane) the LODs were always below 1 ppb, with approximately one order of magnitude better sensitivity. An advantage of LC-MS over GC-ECD was noticed in the determination of 4-nitrophenol and 3,5-dichlorobenzoic acid, which offered derivatization problems under the current EPA method. For this reason, the LC-PB-MS method was recommended for all of the group of analytes. The comparison of the LODs of the various methods used, including also LC-UV, is shown in Table 3.10.

The last method released by the US EPA involving LC-MS incorporates a TSP interface [219]. This is applicable to the determination of a wide range of analytes, including dyes, organophosphorus pesticides, chlorinated acids, and carbamates in waste water, ground water and soil/sediment matrices. This US EPA LC-MS method

TABLE 3.10

COMPARISON OF LODS (ppb) FOR A VARIETY OF ACIDIC CHLORINATED PESTICIDES EXTRACTING 1 l OF WATER SAMPLE WITH GRAPHITIZED CARBON BLACK FOLLOWING DERIVATIZATION WITH DIAZOMETHANE-FOR GC-ECD OR USING HPLC-UV OR HPLC-PB-MS ANALYSIS

Compound	HPLC-UV	GC-ECD	HPLC-PB-MS
Acifluorfen	42	0.1	5.2
Bentazone	9	0.2	0.7
Chloramben	6	0.06	2.6
2,4-D	31	0.2	5.0
2,4,-DB	83	0.2	0.7
Dicamba	9	0.07	4.5
3,5 Dichlorobenzoic acid	14	n.d.	0.7
Dichlorprop	21	0.17	1.5
Dinoseb	15	0.1	1.5
4-Nitrophenol	11	n.d.	1.5
Pentachlorophenol	12	0.05	0.7
Picloram	8	0.1	7.2
2,4,5-T	30	0.8	4.5
2,4,5-TP	25	0.07	1.5

incorporates the longest list of analytes to be determined. In it, the calibration is carried out by using either polyethylene glycol (PEG) 400 or 600. The calibration masses and relative abundances obtained when using PEG 400 are indicated in Table 3.11. The method has been applied widely to the determination of various pesticides including organophosphorus, carbamates, and acidic pesticides, although problems were found with thermally labile pesticides such as trichlorfon when using the TSP interface. The LODs, after the preconcentration of a 1 l water sample generally varied between 0.2 and 5 ppb. This method is more universal than LC-PB-MS although the structural information obtained from LC-TSP-MS is poor compared to PB. The problem with the proposed method is that it includes the analysis of thermally labile pesticides such as trichlorfon, methiocarb and a few others that can easily decompose at the TSP interface temperatures [215]. For this reason, before applying this proposed method it is important to know which are the target pesticides to be determined. From our own experience, the method can easily be applied to chlorinated acids but care should be taken before or when determining the other groups of pesticides.

3.6.4.2. LC-TSP-MS

LC-TSP-MS has been applied to the analysis of a variety of pesticides including carbamates [221–228], organophosphorus compounds [22,229–233], ureas [234], chlorinated phenoxy acids [235], triazines [234,236], and phenolic compounds [237].

In general, in the positive-ion (PI) mode, the protonated molecule $[M + H]^+$, and

TABLE 3.11
CALIBRATION MASSES AND % RELATIVE ABUNDANCES OF PEG 400 UNDER TSP-MS: US
EPA METHOD 8321A

Mass	% Relative abundance
18.0	32.3
35.06	13.5
36.04	40.5
50.06	94.6
77.04	27.0
168.12	5.4
212.14	10.3
256.17	17.6
300.20	27.0
344.22	45.9
388.25	64.9
432.28	100
476.30	94.6
520.33	81.1
564.35	67.6
608.38	32.4
652.41	16.2
653.41	4.1
696.43	8.1
697.44	2.7

The intensity has been normalized to mass 432.

the ammonium adduct ion, $[M + \text{NH}_4]^+$, are most abundant, depending on the proton affinity (PA) of the compounds. In the negative-ion (NI) mode, most often a deprotonated molecule, or an acetate or formate adduct ion is observed. The choice between the positive or negative ion mode depends on the compound. The negative ion mode has been shown to be much more sensitive to rather electronegative compounds such as chlorinated phenoxy acids than is the positive ion mode. The former yields $[M + \text{acetate}]^-$ or $[M + \text{formate}]^-$ base peaks if ammonium acetate or ammonium formate is used as the ionizing additive. In both ion modes, little fragmentation is observed, and thus the structural confirmation of unknown compounds is hampered.

To overcome the limited structural information available with some nebulizer interfaces, coupling with a tandem MS system is interesting. LC-MS-MS permits the characterization of a variety of pesticides [238,239]. In the MS-MS mode a selected ion collides with a collision gas. As a result of the collisionally activated dissociation, the selected ion is fragmented. The use of LC-TSP-MS-MS has been reported for the identification of aquatic photoproducts of chlorotriazine pesticides, obtained after UV-Suntest irradiation which simulates real sunlight conditions [238].

One of the major disadvantages of the use of LC-TSP-MS for pesticide analysis is the degradation of thermally labile pesticides in the probe. Approximately 16% of trichlorfon is converted into dichlorvos. Also, some carbamate pesticides, e.g., methiocarb, suffer thermally assisted degradation at the optimized vaporizer temperatures [223]. The formation of abundant fragment ions by thermally assisted degradation can also be used for compound identification [224]. However, the application of higher vaporizer or ion-source temperatures can cause instability in the spray, resulting in poor reproducibility of the ion signals. Therefore identification can be obtained, although at the expense of quantification data.

In summary, LC-TSP-MS was applied widely for pesticide analysis in the 1980s. Its major drawback was that it gave poor structural information, although this can be solved by using a discharge in the ionization or by using LC-TSP-MS-MS. This second option is the best, although the cost of such instrumentation exceeds the resources of many laboratories. Probably the best verdict on LC-TSP-MS is that this technique, together with LC-PB-MS, permitted the “real” implementation of LC-MS in many research and routine laboratories that are nowadays switching to the new generation of interfaces such as APCI or ESP.

3.6.4.3. LC-PB-MS

Because the particle-beam method offers the possibility of obtaining solvent-independent EI and CI mass spectra, various applications for the determination and identification of carbamate [221,240–242], organophosphorus, triazines, phenylurea, and chlorinated phenoxy acid pesticides have been reported [221,243–247]. EI is considered to be the best understood type of ionization method, and many mass spectra have been reported for the pesticides used. Therefore, the unequivocal identification of an unknown is possibly achieved using this interfacing technique. A disadvantage of EI is that little molecular weight information is obtained. The application of “softer” ionization methods such as chemical ionization utilizing methane, isobutane, or ammonia as reagent gases, can provide both fragmentation and molecular weight information. The use of ammonia CI has resulted in better sensitivity than the other ionization modes, and fragment ions were formed, making the structural confirmation of the compounds possible. Therefore, this ionization mode is of interest when both quantification and identification of the compounds are needed. Its application has been tested and investigated in detail by comparing the mass spectra of 14 selected carbamates obtained from desorption chemical ionization, and ammonia chemical ionization, in PB-MS [240]. The most complete list of carbamates including 33 parent analytes and 14 transformation products was published by Slobodnik et al. [242]. The authors have compared the three modes of operation, EI, positive chemical ionization (PCI), and negative chemical ionization (NCI). It was found that EI performed best for most of the analytes studied as regards analyte detectability. The authors recommended the use of both modes of operation, EI for structure elucidation, and PCI or NCI for molecular weight information. The same group

[241] combined the use of on-line SPE with LC-PB-MS for lowering the LODs of this group of analytes in water samples. After preconcentrating 100 ml of water samples, the LODs for several carbamates varied between 0.1 and 8 ppb, whereas other carbamates such as aldicarb, carbendazim or methomyl exhibited LODs higher than 20 ppb, indicating that LC-PB-MS is not sensitive enough for several polar pesticides; this is one of the limitations of this technique. On-line SPE followed by the LC-PB-MS combination was also applied by Aguilar et al. for the confirmation of a variety of pesticides, from the organophosphorus, phenoxy acid, urea, and triazine classes in surface water samples at levels varying from 0.05 to 0.5 ppb [244]. To enhance the detectability of LC-PB-MS, the enrichment of acidic pesticides into graphitized carbon black permitted the off-line SPE enrichment of 2 l of surface water samples with LODs varying from 0.1 to 1 ppb. The same group has compared its SPE method followed by LC-PB-MS, for the analysis of acidic pesticides, with Method 515.5. The PB-MS method, although less sensitive than the current GC-ECD method, gave good quantitation for all the analytes whereas the current EPA method does not (see Table 3.10) [220].

In general, LC-PB-MS is well established and is used in environmental analysis. Two EPA methods are based on this technique. The most attractive feature of this combination is the possibility of EI spectra and the use of libraries for compound identification. This combination is, however less sensitive than other LC-MS techniques, and calibration problems have been found on many occasions when quantitation was performed. LC-PB-MS has also been found to be compound dependent. To overcome the problems of low sensitivity, the use of on-line SPE permits enrichment of the analytes and reduction of the LODs. Most probably, this combination, even if not improved with regard to sensitivity and discrimination of the analytes in the interface region, will still be used for a while, but after few years it will be replaced by other LC-MS combinations, involving APCI or ESP combined with quadrupoles or ion traps.

3.6.4.4. *LC-ESP-MS*

In this section we discuss two LC-MS coupling techniques: the electrospray (ESP), and high-flow pneumatically assisted electrospray or ion spray (ISP). The nebulization in the ESP and ISP interfaces does not use heat and thus no thermally assisted degradation is expected. The major differences between these two interfaces, for routine pesticide analysis, is that in conventional ESP, only 20–50 $\mu\text{l}/\text{min}$ are directed to the MS source, so it is necessary to split the LC-flow, whereas in ISP up to 0.3–0.4 ml/min can be handled by the ISP interface, with no need for splitting the LC eluent. Several applications to pesticide analysis have been reported in the literature in the last few years [215,221,237,248–258].

Ion-formation in ESP and ISP is due to the evaporation of ions from charged droplets, generating rather cold ions with nearly no excess internal energy, and thus little or no fragmentation is observed. For the enhancement of the sampling efficiency of the ions formed, an extraction potential is applied, focusing the ion spray

towards the entrance orifice. An increase in this voltage will increase the total ion current. Moreover, higher voltages have been proved to induce fragmentation of various types of analytes, which is comparable to the fragmentation observed in collisionally activated dissociation (CID) mass spectra [248].

One of the most notable aspects of LC-ESP or ISP-MS is the formation of the $[M + Na]^+$ ion. The sodium ions present originated from impurities in the methanol solution. Since under ISP-MS the vaporization takes place from the solution, it is reported that 90% of the observed ions are from ions present in the solution. The second point arises from the use of methanol or acetonitrile in the mobile phase. Acetonitrile certainly reduces the abundance of the sodium-cationized ions versus methanol, as reported for various organophosphorus pesticides, monuron and carbofuran [251]. The sodium ion addition is thermodynamically favoured and occurs preferentially in dilute solution, but in more concentrated solutions the sodium ions are depleted and protonation becomes dominant. In general, we can say that the formation of abundant adduct ions with sodium is related to a number of factors. The first is the use of methanol in the mobile phase. Secondly, they are compound-dependent, as reported in our own work which showed very low abundances of the sodium adduct ion for triazine herbicides whereas for phenylurea the sodium adduct ion was the base peak [250]. Thirdly, they are dependent on the concentration of the compound and on the cone voltage used. In this respect it was noticed that the $[M + Na]^+$ ions can exhibit high or low abundances versus the increased cone voltage. In this respect, the $[M + Na]^+$ ion was stable for phenylurea herbicides, and it was impossible to obtain any fragmentation even at a cone voltage of 100 V. This behaviour was totally different from the triazine herbicides and was attributed to the stability of the aromatic structure of the phenylurea herbicides with the sodium adduct ion, as compared to the triazines [250]. Again, the fact that the $[M + Na]^+$ ion is stable under different extraction voltages is compound-dependent. If additives such as NH_4^+ or H^+ are added to the eluent then the signal-intensity of the sodium adduct ions diminishes sharply.

The formation of adduct ions others than $[M + H]^+$ is not very favourable, because the variations of the salt content in the organic solvent can reflect the variations of the abundances of both fragment and parent ions. The addition of an acidifying agent into the mobile phase is frequently not enough to suppress the $[M + Na]^+$ ion, so an elegant solution proposed by Crescenzi et al. was to use a prior distillation step for eliminating sodium and other cations from the organic solvent [256].

The major attraction of LC-ESP-MS as applied to pesticide analysis is its low detection limits for determining pesticides in water when it is combined with SPE. The LODs for organophosphorus pesticides varied from 0.01 to 0.2 ppb, using only 200–300 ml of water after off-line SPE using disks or cartridges [249,251]. An example of the analysis of various thermally labile carbamates showed that it was possible to determine them by direct analysis at the 0.1 ppb level in water, by using a large volume injection of 0.5 ml of water [253].

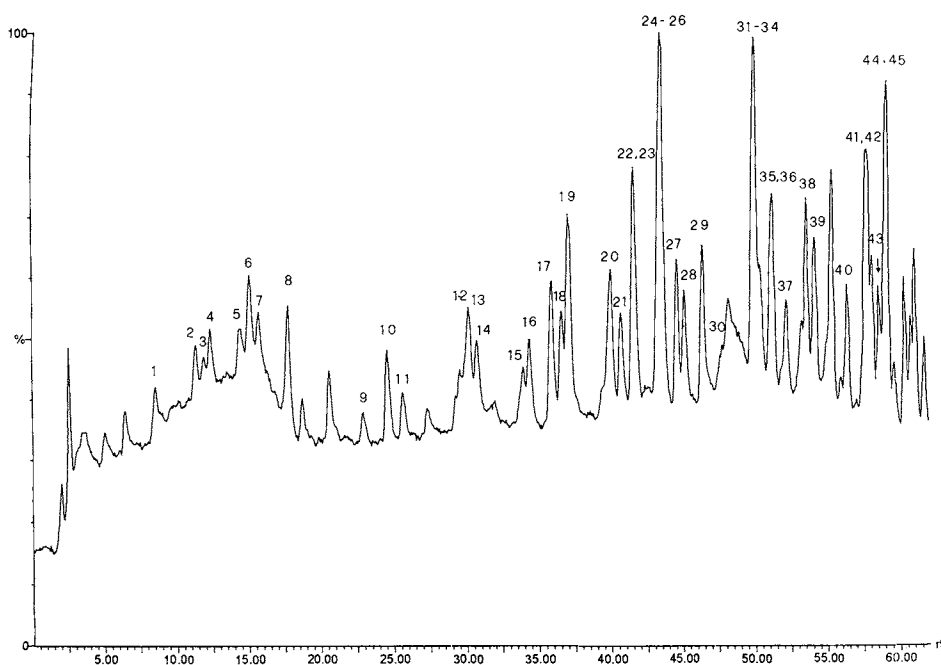


Fig. 3.13. Full scan LC-ESP-MS chromatogram obtained by analyzing 1 l of river Taber water sample spiked with 45 pesticides at the individual level of 200 ng/l. Peak numbers: (1) omethoate; (2) butoxy-carboxim; (3) aldicarb sulfone; (4) oxamyl; (5) demeton sulfoxide; (6) methomyl; (7) demeton sulfone; (8) monocrotophos; (9) trichlorfon; (10) dimethoate; (11) chloridazon; (12) carbendazim; (13) butocarboxim; (14) aldicarb; (15) carbetamide; (16) cyanazine; (17) propoxur; (18) carbofuran; (19) simazine; (20) carbaryl; (21) ethiofencarb; (22) pirimicarb; (23) monolinuron; (24) chlorotoluron; (25) atrazine; (26) metazachlor; (27) isoproturon; (28) methabenzthiazuron; (29) diuron; (30) azinphos-methyl; (31) tertbutylazine; (32) mercaptodimethur; (33) linuron; (34) propanil; (35) malathion; (36) propyzamide; (37) molinate; (38) ethoprophos; (39) metolachlor; (40) neburon; (41) propiconazole; (42) diazinon; (43) phoxim; (44) pirimiphos methyl and (45) prochloraz. Reproduced from Ref. [256] with permission.

The fragmentation ions found for most of the pesticides studied, such as organophosphorus compounds [249,251], carbamates [253], triazines [250,257], phenoxyacids [254,255] and multiresidue methods involving 45 pesticides [256], gave results in agreement with those reported for GC-MS. Typical diagnostic ions were generally obtained. This is a very important feature of ESP since it is a technique that can be used for the identification of unknown pesticides, unlike most of the TSP applications that usually give very poor structural information. Consequently, it is used for confirmation purposes with target analytes.

The combination of SPE followed by LC-ESP-MS has been demonstrated recently by analyzing 45 pesticides spiked in river water samples. Figure 3.13 shows the full scan chromatogram of 1 l of river water sample spiked with the 45 pesticides

at the 200 ng/l level. The sample was analyzed during 1 day, and seven extracts were injected one after the other, the repeatability of the system being better than 10%.

In summary we can say that LC-ESP-MS and LC-ISP-MS are each very useful techniques for analyzing pesticides in water, after SPE. These combinations give enough structural information, and increasing the extraction voltage of the system permits the identification of unknown analytes. However, the most relevant feature is their sensitivity which permits one to use less than 1 l of water samples (200–500 ml) and reach LODs below 0.1 ppb, which can be used for compliance with European Union directives for drinking water. Although the ESP or ISP techniques are very sensitive for many pesticides, they still cannot be considered universal. In this respect, pesticides from the so-called parathion group, such as parathion or fenitrothion, and also other pesticides such as DDT [249,256] did not produce sufficient gas phase ions and will require the use of other LC-MS approaches. Something similar was noticed for phenolic compounds. The most polar phenols could only be analyzed by ISP, whereas most of the other phenols were determined by LC-APCI-MS [237]. Overall, ESP techniques will be increasingly used for environmental analysis owing to their enormous potential as regards sensitivity and their power for the identification of polar and ionic unknown pesticides and metabolites.

3.6.4.5. LC-APCI-MS

When LC-APCI-MS is used, the ionization process involves gas phase ion molecule reactions, which cause the ionization of the analyte molecules under atmospheric pressure conditions. Under APCI-MS, both heat and pneumatic nebulization are applied to evaporate the sample solution and to obtain the spray. APCI-MS is expected to be less dependent on the pre-ionization of the analytes in the sample solution and to be more suitable for the determination of compounds covering a wide polarity range, including polar and medium or even non-polar analytes. LC-APCI-MS can usually handle flow rates up to 1.5 ml/min. In the last few years, several applications to the determination of pesticides in water matrices have been reported [20,221,237,259–264]. Under APCI-MS conditions, the use of higher cone voltages permits, as with ESP, enhancement of the mass spectral structural information and the achievement of good confirmation of analytes. The first comment to be made is that, in general, more fragmentation is obtained, under APCI-MS than with the previous ESP. This could be observed for various organophosphorus pesticides by comparing the use of LC-ISP-MS [249] or LC-APCI-MS [20]. Another advantage of APCI versus ISP for analyzing organophosphorus pesticides, is that various organophosphorus pesticides such as fenitrothion, chlorpyrifos and fenchlorphos, were not detected at low levels under ISP, whereas they exhibited good sensitivity under APCI [20,249,261]. This is related to the fact that these compounds need heat in addition to the pneumatic nebulization for the ionization of their molecules. Another advantage of APCI is that it produces fewer sodium and solvent cluster ions than does ISP, when using the same type of methanol.

As is the case with LC-ESP-MS, the on-line SPE coupling with LC-APCI-MS is also feasible. By using such a device, it is possible to determine at the ppt level most of the pesticides analyzed by preconcentrating only 100–200 ml of water. Figure 3.14 shows the PI- and NI LC-APCI-MS chromatograms obtained under SIM conditions after preconcentrating 200 ml of a drinking water sample. Some of the analytes are better determined under PI and others are detected by the NI mode, depending on the types of analyte and their gas phase chemical properties [264]. In this respect, APCI has similarities with GC-MS using chemical ionization. Another interesting feature as we compare APCI with ESP is that LC-APCI-MS under the NI mode is much easier to perform than LC-ESP-MS with NI. In the latter case, it is necessary first to add acid to the mobile phase for the LC separation of acidic herbicides and later on, after post-column elution, to add a neutralization buffer to form ions in solution and to facilitate better charging of droplets [254].

Finally, the use of LC with a short column combined with APCI-MS-MS was reported to be a good alternative for the fast determination of 17 pesticides in water matrices, achieving successful identification since the MS-MS mode can be used and library identification is guaranteed [261].

In summary, we should indicate that LC-API-MS will be the method of choice for most environmental applications, mainly because of its ease of use, since it can be combined with most available LC equipment using conventional flow rates. Using either PI or NI, the characterization of a large number of analytes is feasible. The combination with either off-line or on-line SPE devices permits automation of the determination of pesticides in water matrices and achieves LODs which comply with the most stringent regulations on pesticide residues in water matrices.

3.6.4.6. *Future trends*

The use of LC-MS techniques in pesticide analysis has grown considerably in recent years, and will be increasingly used. The major point is that in the last few years many MS manufacturers have developed the so-called bench tops, LC-MS machines incorporating either API or ESP sources. Such devices, with prices in the range of US\$130 000–150 000, are very competitive and are being purchased by an increasing number of research and routine laboratories. Certainly, the advent of APCI and ESP-MS interfacing systems have expanded the applicability of LC-MS in environmental analysis, mainly because of the high sensitivity and structural information obtained. Nowadays it is accepted that these two interfaces are the most applicable interfaces in the environmental area and can, in a way, be considered the standard interfaces for environmental analysis. The identification potential of LC-ESP-MS can be enhanced by using an ion trap, which has several commercial manufacturers, or by using a MS-MS system. The use of ion-trap MS has a price still lower than tandem MS, but it is more expensive than a single quadrupole instrument of the so-called bench top type. Developments in the area of LC-ESP-MS with ion-trap source will need to take place, especially with regard

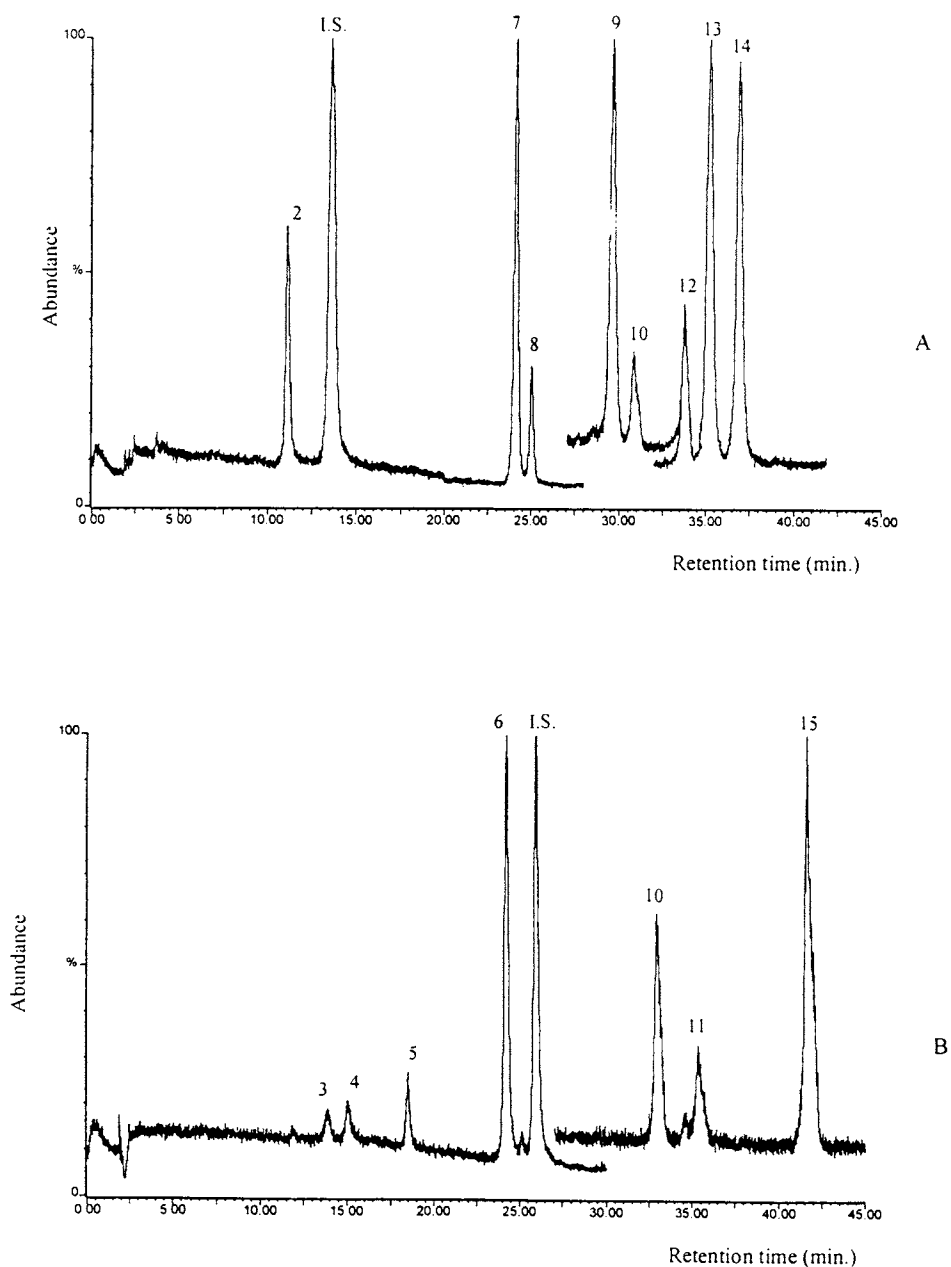


Fig. 3.14. On-line SPE followed by LC-APCI-MS SIM chromatogram obtained by preconcentrating 200 ml of drinking water sample spiked with 15 pesticides at the individual level 40 ng/l under PI (A) and NI (B) modes of operation. Peak numbers: (1) bentazone; (2) vamidothion; (3) 4-nitrophenol; (4) MCPA; (5) Mecoprop; (6) Dinoseb; (7) atrazine; (8) isoproturon; (9) ametryn; (10) malathion; (11) fenitrothion; (12) molinate; (13) prometryn; (14) terbutryn and (15) parathion-ethyl.

to the quantitation of analytes, since this problem is not completely solved in current ion-trap MS.

3.6.5. CE-MS

Another new technique in this field is the use of capillary electrophoresis (CE)-MS which permits the separation of ionic compounds in an open tube, through the application of a voltage gradient. Compounds migrate through the tube differentially as a result of their electrophoretic mobility. As an on-line separation method, CE-MS distinguishes analytes by their differences in electrophoretic mobilities and by giving structural information. CE-MS combines the advantages of CE and MS so that information on both high-efficiency separation and molecular masses and/or fragmentation can be obtained. Other advantages are that no heat or pumping of solvent is required; the ionization is at ambient temperature and is mild, thus making the process suitable for labile analytes and the instrumentation is simple. Several ionization methods have been used for CE-MS: electrospray, ion spray, and continuous-flow fast-atom bombardment (FAB). A book chapter [215] and review article [265] have been published. Various applications have been reported in the literature on the determination of pesticides in water matrices [215,265–268]. Several of these applications are common to applications of CE-UV detection. The analysis of quaternary ammonium herbicides that are commonly analyzed by CE, such as meququat, chlormequat, diquat, paraquat and difenzoquat, has also been reported by CE-MS and CE-MS-MS [266,267]. A volatile electrolyte solution containing acetic acid–ammonium acetate with 10% methanol was used as modifier, to reduce adsorption of the solutes on to the capillary wall and to be compatible with MS. Under CE-MS most of the quats gave $[\text{Cat}]^+$, whereas when using CE-MS-MS, abundant structural information was obtained. The LODs in analyses of drinking water matrices varied from 10 to 70 ppb. The US EPA has also published a paper on the analysis of diquat and paraquat by means of CE-MS, although it is not yet an official US EPA method [266]. In the USA, the maximum contaminant level for these two analytes in drinking water is 20 ppb and such compounds are being analyzed by the US EPA method 549.1 (see Table 3.5) which permits their determination at the 0.4–0.8 ppb level, after SPE using 250 ml of water and LC separation with DAD. The development of a method involving MS will solve the problems of final confirmation of these two analytes in environmental matrices. In this way Song and Budde [266] have used CE-MS with a separation buffer of 0.5–1.5% acetic acid in 50% methanol–water and with a second electrolyte at 5 mM. Various electrolytes were tested: ammonium bicarbonate, ammonium acetate, ammonium formate and ammonium citrate. It was observed that with ammonium bicarbonate as the separation buffer electrolyte, the M^{2+} ions and deprotonated ions have about the same abundance. When using ammonium acetate, formate and citrate, the deprotonated ions become the base peaks. The smallest amount of diquat and paraquat that could be determined was 200 ppb, which is still

higher than the US EPA regulations. These LODs are somewhat higher than those obtained under CE-UV [156,158]. We should point out that no sample pretreatment was performed, so with an enrichment factor of 100 it will be feasible to determine both analytes at the 2 ppb level, which will be acceptable for the US drinking water regulations.

An application of partial-filling MEKC and ESP-MS was demonstrated for the analysis of triazine herbicides [268]. This technique involves filling a small portion of the capillary with a sodium dodecyl sulfate micellar solution for achieving the separation. After the injection into the system, the triazine analytes migrate first into the micellar plug where the separation occurs and then into the electrophoresis buffer which is free of surfactant. Consequently the electro-osmotic transfer of neutral triazines to the ESP-MS at the end of the MEKC capillary is comparable to that in conventional capillary zone electrophoresis ESP-MS. For this reason, such a novel approach provides a way for the separation and mass-detection of neutral molecules without interference from the surfactant.

In summary, CE-MS is an emerging technique for the analysis of amphoteric or ionic pesticides. Its major limitation is its relatively poor concentration sensitivity. For this reason, prior enrichment techniques will be needed for pesticide analysis, which in a way is also common to LC and GC. The most popular application so far in the analysis of pesticides is in the determination of "quats", which are a group of pesticides, very difficult to analyze by GC or LC techniques; in this case, CE has proved to be a powerful and sensitive method. In the coming years, much more development of the coupling of CE with MS will certainly be conducted, and more environmental applications will be published.

3.6.6. Miscellaneous

Under this heading we indicate a few coupling techniques based on MS or spectrometric techniques that have been published recently and that should receive some attention. FAB-MS has scarcely been used in environmental analysis, although the analysis of atrazine and hydroxyatrazine in agricultural run-off water has been published [269]. SPE with carbon black was used for extraction of the two analytes from water samples, and afterwards the simultaneous determination of both analytes was carried out by FAB-high-resolution MS. The concentrations of the analytes were measured at levels varying from 0.2 up to 15 ppb. The coupling of on-line SPE followed by LC with Fourier transform infrared spectrometry (FT-IR) was carried out by Somsen et al. [270]. Using 50–100 ml of water sample, identifiable spectra of triazines and phenylurea were feasible in water matrices, with an identification limit of 1 ppb. A peak search routine which is based on the matching of peak frequencies alone was found to be the most suitable for identifying analytes at trace levels. Although the method is not as sensitive as the coupling with LC-MS, it is very promising, and at least permits the detection and identification of pesticides at the so

called “alarm” level for pesticides in European surface waters. Laser-disruption FT-ion-cyclotron MS (LD-FTICR-MS) was coupled with SPE on Empore disks for the determination of flumequine, isoproturon and flutriafol in water matrices [271]. The three pesticides investigated easily produced characteristic spectra by LD-FTICR-MS at 248 nm. When absorbed onto Empore disks, only flumequine was detected at 248 nm, whereas detection at 345 nm was needed for isoproturon and flutriafol. Characteristic fragments for each pesticide were obtained and the LODs were in the range of 0.1–1 ppm. The same pesticides were investigated by the same group by determining the pesticides absorbed onto SPE Empore disks by matrix-assisted laser desorption ionization (MALDI) combined with a quadrupole ion trap MS [272]. The MS-MS capabilities of the ion trap allowed the structural elucidation of the target pesticides. The SPE membranes loaded with non-volatile pesticides and coated with an appropriate matrix were laser desorbed to assess the effectiveness of the MALDI ion-trap approach. LODs obtained in this study were in the range of 25 ppb, but the possibility of greater sensitivity has been established using the ion-accumulation procedures of the ion-trap. Preliminary investigations suggest that this technique may be used for quantitative purposes in the future.

3.7. CONCLUSIONS

In this chapter we have described the various chromatographic methods commonly used for the determination of pesticides in water matrices. GC is still the method of choice for analyzing a wide range of pesticides, and the simplest approach only requires two detectors, mainly ECD and NPD, and two columns of different polarities. With such simple devices many pesticides can be determined and confirmed by a secondary column. For this purpose, the EPA methods have been presented and give basic guidelines for the determination of pesticides.

LC techniques have been used widely during the last 10 years for pesticide analysis. There are various advantages that have been already mentioned in this chapter that make the technique attractive for pesticide analysis. The use of diode array detection, the most universal detector for pesticides, which also gives some kind of structural confirmation by UV spectra, is the most desirable approach. Several EPA methods have been released using LC, and the technique is continuously growing, especially because it permits automation and the on-line SPE enrichment of samples. An old technique, thin layer chromatography was also discussed, although it is perhaps the least used analytical method for pesticides at this moment. Indeed, with the current prices of the GC instrumentation and the development in capillary GC with selective detectors, GC will be preferred over thin layer chromatography as a routine technique. In the past, thin layer chromatography was a method of choice for pesticides because GC and especially LC methods, were not fully developed or were rather expensive.

CE is an emerging technique for the analysis of very polar and ionic pesticides

which are difficult by GC – most of them will require derivatization – or by LC. The development of CE will continue in the next few years and it is expected that different analytical methods for the routine analysis of pesticides in water will be developed. The sensitivity of this technique for environmental analysis is still its major drawback, but the technique has definitely found its place in pesticide analysis.

An increase in the use and development of MS techniques has been taking place in the last few years for the detection of environmentally important compounds. GC-MS with EI and chemical ionization is of great help for confirmation and/or analysis of many pesticides at the low ppb level in water samples. GC-MS accomplishes the main points desired in an analytical technique: selectivity, which can be enhanced by the use of SIM and NCI, sensitivity, and reproducibility. Because of the evident needs for confirmation and/or quantitation, monitoring programmes are incorporating GC-MS, namely bench tops using EI, to confirm unequivocally the different pollutants. The main characteristics of GC-MS with EI are its convenience, its easy use for daily work, day-to-day reproducibility, the possibility of library searches, and sufficient structural elucidation. In the last few years, GC-MS using bench top instrumentation has expanded the range of options, and many of them are now available with PCI and NCI. No doubt, the applications of GC-MS with NCI, which offer good selectivity and sensitivity for specific applications, will grow in the future. The development of GC-ion-trap MS with the possibility of MS-MS characterization of the analytes also expands the possibilities of this technique, and at a more reasonable price that can be afforded for more laboratories involved in research and routine pesticide analysis. The on-line coupling of systems involving SPME will be used more, and routinely applied for determining a great variety of pesticides in water matrices. They are easy to use, have a low cost, and permit one to perform multiresidue analysis in aqueous matrices in a relatively short time, with reduced sample manipulation.

LC-MS techniques have gained a good reputation in the last few years for the determination of pesticides. In addition to the advantages of LC techniques, the use of MS permits the unequivocal confirmation of pesticides. With the implementation of the APCI and ESP interfaces, it is now possible to achieve sensitivity limits that approach those of GC and also permit screening for unknown pesticides in water samples, because of the increased sensitivity of these ionization techniques over PB and/or TSP. During the next few years LC-APCI-MS will be used in a similar way to GC-MS in many laboratories and probably this technique, possibly with improvements in the design, will remain as the LC-MS method of choice for pesticides.

CE-MS still needs to be developed further for environmental analysis, especially with respect to the final configuration of the coupling, the reproducibility of the system, and sensitivity. However, this technique will grow in the coming years since many applications of CE are being reported, to a great variety of environmental pollutants. Instrumentation developments are still needed in this area, in a similar way to the developments in LC-MS interface coupling which took place 10 years ago. CE-

MS will have its role in pesticide analysis and will cover analyses of, for example, “quats”, which offer difficulties under LC-MS.

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CHAPTER 4

Sample Handling Techniques (Extraction and Clean-up of Samples)

4.1. INTRODUCTION

Many environmental samples cannot be analyzed without preliminary sample preparation because they are too dilute or the matrix is too complex. Preconcentration of samples of relatively large volumes (in the range 100–1000 ml or more) is often necessary to overcome the limitation of the detection systems, but the extract is often too complex for efficient separation by the chromatographic column at low detection levels, so that additional treatment is required before separation. The objective of the sample pretreatment is to provide a sample fraction enriched in all the pesticides of interest, and as free as possible from other matrix components. This pretreatment, which can be achieved in one to three different steps, consists of (i) extracting traces of analytes of interest from the aqueous media, (ii) concentrating these traces, (iii) removing from the matrix other components which have been co-extracted and co-concentrated and which may interfere in the chromatographic analysis (i.e., clean-up).

Before implementing any strategy, it is important to consider the strong interdependence of the various steps of the whole analytical procedure, i.e., sample handling, separation and detection. There is no unique strategy for the pretreatment of samples of pesticides in waters. It mainly depends on the nature of the solutes to be determined (e.g., volatility or polarity), on the nature of the matrix, and on the level of concentration required. Interference removal is a critical step which is strongly related to the concentration of the analytes of interest and on the nature of the aqueous media. In other words, the strategy for determining a pesticide below the microgram per litre level in drinking water will be different from that used in very polluted river water. It will also be guided by the separation, and especially by the detection mode. If a very selective detection can be carried out, the sample handling may be simplified. On the other hand, if a selective trace-enrichment is applied, a simple detection mode can be used and the clean-up is no longer required, even for complex

matrices. This "total system" approach is of prime importance for selecting the optimal sample-handling strategy [1].

Despite the advances in separation and quantification techniques, the sample pretreatment is still the weakest link and the time-determining step in the whole analytical procedure, accounting for about the two-thirds of the total analysis time and it is also the primary cause of errors and discrepancy between laboratories [2–4]. In the environmental literature, and in many official methods, most of the sample preparations described are based on manual and time-consuming procedures that have been used for decades and consume large amounts of toxic organic solvents. There is a need for the development of sample-handling strategies which are faster, more reliable (which means that the number of intermediate steps, such as transfers, evaporation and derivatization is diminished) and easily capable of automation and are as solvent-free as possible. There is general agreement on the following observations: (i) in every country and at each level, analytical chemists receive a training in separation methods, but not in sample preparation; (ii) the sample preparation is still not well considered and is certainly not recognized as the most important step of the analysis scheme -it is often given to the least trained technician; and (iii) many extraction methods are poorly developed, with little consideration being given to the chemistry involved.

The methods for extraction and concentration of pesticides are mainly liquid-liquid extraction (LLE) and solid-phase extraction (SPE). The technique which is still often preferred by many environmental chemists, especially as the first approach, is LLE. This is because most of the official methods use it for its simplicity [5,6]. LLE is a fully developed technique, well described in any environmental literature, and applications to pesticides are reviewed briefly in this chapter. In recent years, enrichment of trace compounds on suitable sorbents has been shown to be an interesting alternative to LLE, and has now become a reliable and useful tool for sample handling. Because recent regulatory pressures require a reduction in the amount of organic solvents used in analytical laboratories, government regulation agencies are becoming quite flexible in accepting SPE. In addition, many modern pesticides and identified degradation products are fairly soluble in water and are therefore less amenable to solvent extraction. The scope of SPE for automation, for on-line coupling with the chromatographic separation, and the possibilities for carrying out very selective preconcentrations are the main reasons for further developments.

Trace-enrichment techniques are commonly used off-line. Pretreatment steps are therefore clearly separated from the chromatographic separation. Automation is possible, especially for SPE techniques, using robotics or special sample preparation units that sequentially extract the samples and clean them up for further injections. Automatic devices which couple on-line the sample pretreatment by SPE and the liquid- or gas chromatographic separation have recently been introduced by some companies. This represents a fast, modern and reliable approach for monitoring pesticides in water which is described in the next chapter.

In this chapter, we first describe the general aspects of the various methods. The chemical principles which govern the process of the extraction are given. Emphasis is also given to new techniques which reduce the use of organic solvents- such as micro liquid-liquid extraction, already recommended in some US Environmental Protection Agency (EPA) methods [6], or supported liquid membrane extraction procedures [7-10]. A major part is devoted to SPE techniques which are based on LC principles and which therefore appear less straightforward than LLE to environmental chemists who are not familiar with LC techniques. A comprehensive description of the main principles governing SPE and the prediction of the SPE parameters (selection of the sorbent, recoveries, sample volume, etc.), is therefore given according to the physico-chemical characteristics of the major pesticides and their transformation products. The performance of recently available polymeric sorbents with high specific areas, and of carbon-based sorbents are presented with special attention to their ability for extracting some polar and water-soluble pesticides or transformation products. New methods involving solid-phase microextraction (SPME) and supercritical fluid extraction (SFE) techniques are also presented. SFE is a promising technique for analyzing the partitioning of pesticides between the dissolved and the suspended phases which occur in natural water samples. Many examples are given, especially of multiresidue determination, along with the limits of detection obtained in real water samples without further clean-up. Attention is also given to selective sorbents such as immunosorbents which are now under development.

In the last part of this chapter, the various methods for cleaning up the extracts are studied, based on solid-phase extraction with polar sorbents such as silicas, aluminas or Florisil, or on gel permeation chromatography.

Attention is also given to the automation of parts or of the whole sequence of the sample handling.

4.2. EXTRACTION AND CONCENTRATION PROCEDURES

4.2.1. Liquid-liquid extraction (LLE)

4.2.1.1. General considerations and basic parameters

Liquid-liquid extraction is based on the partition of organic compounds between the aqueous sample and an immiscible organic solvent. The efficiency of an extracting solvent depends on the affinity of the compound for this solvent, as measured by the partition coefficient, on the volume ratio of each phase, and on the number of extraction steps.

Solvent selection for the extraction of environmental samples has been described in many reviews [11-15] or in official methods, and is related to the nature of the pesticides. The large choice of available pure solvents, which provide a wide range of solubility and selective properties, is often claimed as an inherent advantage of LLE techniques. In fact, each solvent is seldom totally specific towards a given class

of compounds and LLE is mainly used for the wide spectrum of compounds extracted [16]. Apolar and slightly polar solvents are generally chosen. Hexane and cyclohexane are typical solvents for extracting non-polar compounds such as organochlorinated or some organophosphorus pesticides [17]. Dichloromethane and chloroform are certainly the most common solvents for extracting non-polar to medium-polarity pesticides [18]. Table 4.1 summarizes the main EPA methods using LLE and dealing with analysis of pesticides in water. The typical sample volume is 1 l, except for methods 504 and 505 which involve a microextraction [5,19,20]. The EPA accepts recoveries in these methods between 70 and 130%. The detection limits are in agreement with the Health Advisory Levels and drinking water regulations from the Office of Water of the US EPA. Many European laboratories used LLE methods which are derived from or are similar to those described in Table 4.1. The enrichment factor can be increased by reducing the final volume (set at 5 ml in many EPA methods) and the detection limits can therefore be closer to the EU regulatory levels of $0.1 \mu\text{g/l}$ for each pesticide in drinking water. It is important to note the trends shown by the EPA for reducing the consumption of organic solvents by carrying out micro-extractions. Only 2 ml of dichloromethane is required in methods 504 and 505. Such micro-LLE can allow quantification at the $0.1 \mu\text{g/l}$ level for some specific compounds, as shown in method 504. Atrazine and six acetanilide herbicides and metabolites were rapidly determined in ground water in the $0.1\text{--}2.5 \mu\text{g/l}$ range using a one-step extraction of water samples (60 ml) with 1 ml of hexane, followed by direct analysis of extracts using capillary GC with nitrogen-phosphorus detection or combined GC-MS [21]. A rapid micro-LLE was also described for trace analysis of organic contaminants in ground and drinking water [22]. Another example is the determination of organochlorine and pyrethroid insecticides extracted by 10 ml of hexane for 15 ml of water samples and further concentrated to 1 ml. After an automated clean-up, such a micro-extraction allows one to analyze a group of 18 organochlorine pesticides and the main pyrethroid insecticides in surface waters at the ng/l level [23]. In the EPA method 507, organophosphorus pesticides were extracted with dichloromethane. A similar method for determining the organophosphorus pesticides has been described in the Official Methods of the Department of Environment Drinking Water Inspectorate Standing Committee of Analysis in the UK (SCA methods). The difference from the method 507 is that the method included some non-polar organophosphorus pesticides, so that for 1 l of drinking or river water the method used 25 ml of hexane and 50 ml of dichloromethane which allowed better recovery of the more non-polar organophosphorus pesticides such as chlorpyrifos, fenitrothion, carbophenothion and pirimifos-methyl. The LOD varied between 0.04 and $0.8 \mu\text{g/l}$. Synthetic pyrethroids and other non-polar pesticides, such as permethrin, cypermethrin, alphacypermethrin, fenvalerate and deltamethrin were also extracted with 100 ml of hexane for 1 l of water with further clean-up and analysis by GC-ECD. Limits of detection were $0.005 \mu\text{g/l}$ for each analyte, thus complying with EU directives [24].

TABLE 4.1
SUMMARY OF THE SAMPLE PRETREATMENT USED IN EPA METHODS FOR PESTICIDE ANALYSIS IN WATER AND BASED ON LLE (FROM REF. [6])

Method and pesticides of interest	Extraction conditions	Analysis conditions	Estimated detection limits ($\mu\text{g/l}$)
<i>EPA method 507</i> Determination of 46 nitrogen- and phosphorus-containing pesticides in water	1 l of water extracted with dichloromethane by shaking in a separatory funnel; the extract is isolated and concentrated to a volume of 5 ml after solvent substitution with methyl tert butyl ether (MTBE)	GC-NPD	0.1–4.5
<i>EPA method 508</i> Determination of chlorinated pesticides in ground water	1 l of water extracted with dichloromethane by shaking in a separatory funnel; the extract is isolated and concentrated to a volume of 5 ml after solvent substitution with methyl tert butyl ether (MTBE)	GC-ECD	0.02–5
<i>EPA method 515.1</i> Determination of chlorinated acids in ground water	1 l of water adjusted to pH 12 and shaken for 1 h to hydrolyze derivatives; extraneous inorganic material is removed by solvent wash; the sample is acidified and the chlorinated compounds extracted with diethyl ether; the acids are converted to their methyl esters using diazomethane	GC-ECD	0.02–1.3
<i>EPA method 505</i> Determination of organohalide pesticides in water	35 ml of sample are extracted with 2 ml of dichloromethane	GC-ECD	0.08–7
<i>EPA method 504</i> Determination of 1,2-dibromoethane and 1,2-dibromo-3-chloropropane in water	35 ml of sample are extracted with 2 ml of hexane	GC-ECD	0.01

With 1 l of water sample and three extractions with a total volume of 200 ml of dichloromethane, average extraction recoveries obtained for about 30 commonly applied medium-polarity pesticides were 60–90% [25,26]. Extraction recoveries may depend on the spiking level and were found to be higher when samples were spiked with 200 ng/l instead of 50 ng/l [26]. They may also be slightly different when measured in spiked pure water samples or in real samples.

LLE can be performed simply, and batchwise, using separatory funnels. The partition coefficient should therefore be large because there is a practical limit to the phase volume ratio and the number of extractions. When the partition coefficient is small and the sample very dilute, a large volume is to be handled and continuous liquid-liquid extractors should be used. The extractions then take several hours. Such extractors have been described in the literature [12,27–29].

The LLE performed as described above is non selective. The so-called lipid fraction is obtained by extraction with chloroform and contains many organic compounds such as alkanes, aliphatic and aromatic hydrocarbons, alcohols, fatty acids, and sterols, in addition to organic micropollutants. The partition coefficient may be increased by adjusting the pH to prevent ionization of acids or bases, by forming ion-pairs or hydrophobic complexes (with metal ions for instance). One example is shown in Table 4.1 for the extraction of the chlorinated acids in the method EPA 515.1. The solubility of analytes in the aqueous phase can be reduced by adding salts.

Fractionation of samples into acidic pesticides and basic/neutral fractions is obtained with successive extractions at different values of pH. A typical scheme is given in Fig. 4.1. which is based on the scheme given for the analysis of EPA priority pollutants lists. Such a type of fractionation was applied for the determination of pentachlorophenol in contaminated waters. No further clean-up of the acidic fraction was needed and pentachlorophenol was determined by GC using electron-capture detection after simple methylation [30].

It is generally impossible to analyze field samples immediately after collection and sample extraction. Munch and Frebis [31] have studied the stability of well water samples and final extracts for the 147 pesticides contained in the National Pesticide Survey list, using the EPA methods described in Table 4.1. Up to 100% loss was observed for 26 compounds spiked in well water samples which had been biologically inhibited and stored at 4°C for 14 days, but analytes generally remained stable in stored sample extracts. More details about stabilization techniques are given in Chapter 2.

4.2.1.2. Limitation of LLE techniques to polar pesticides and/or degradation products

The LLE of relatively polar and water-soluble organic compounds is, in general, difficult. As an indication, the recovery obtained from 1 l of water with the appropriate amount of dichloromethane was 90% for atrazine but lower for its more polar degradation extracted products, de-isopropyl- (16%) de-ethyl- (46%) and hydroxy-atrazine (46%). By carrying out the LLE with a mixture of dichloromethane and ethyl acetate with 0.2 M ammonium formate the extraction recoveries were increased to 62, 87 and 65% for the three degradation products respectively [32]. Ethyl acetate is increasingly used as a replacement for chlorinated solvents. Table 4.2 shows the recoveries obtained for various polar pesticides, classified in order of increasing polarity [33]. Many transformation products which are more polar than the parent

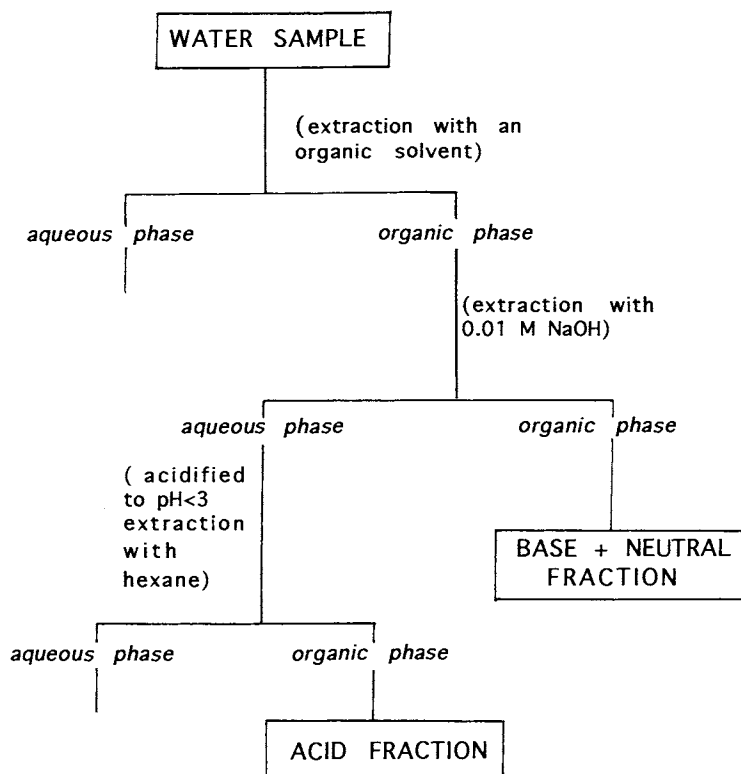


Fig. 4.1. Typical scheme for fractionating water samples for the simultaneous extraction of acidic and base/neutral pesticides.

molecule such as aldicarb sulfone and sulfoxide, de-isopropylatrazine, methiocarb-sulfoxide and -sulfone, and 8-hydroxybentazone are extracted with recoveries below 50%.

Micellar extraction has recently been evaluated as a preconcentration step with the herbicide napromide from ultrapure water and natural samples [34]. The use of aqueous surfactants turned out to be a simple, safe, inexpensive and non-polluting method for extracting and enriching napropamide from a water sample in one step. The extent of the preconcentration was influenced by experimental parameters such as the surfactant and salt concentration. The lower the initial concentration and the more electrolyte is used, the higher the enrichment factor that can be obtained. Two non-ionic surfactants Genapol X 80 and X 150 can be used but Genapol X 80 is preferred owing to its shorter extraction time. When this was combined with the fluorescent detection of napromide within the micellar phase it was possible to achieve detection limits below $0.2 \mu\text{g/l}$. The results demonstrated the usefulness of the cloud-point extraction system.

4.2.1.3. Concentration procedures

LLE results in the extraction of the sample into a relatively large volume of solvent which has to be concentrated down to a few millilitres using a rotary evaporator, or a Kuderna-Danish evaporative concentrator, or some other automated evaporative concentrator. Further concentration to a few hundred microlitres can be obtained by passing a gentle stream of pure gas over the surface of the extract contained in a small conical vessel. The solvent-evaporation method should be slow and the risk of contamination exists. The enrichment factors can be as high as 1000–5000 when handling a 1 l sample and obtaining an extract of 200–1000 μ l. Microextractors have been described and have the advantage of avoiding the further concentration of organic solvents [11,35]. One of these allows the handling of an aque-

TABLE 4.2
AVERAGE PERCENT RECOVERIES AND COEFFICIENT OF VARIATION (CV, $N = 6$) OF
PESTICIDES SPIKED IN RHONE RIVER WATER USING LLE WITH METHYLENE CHLORIDE
(FROM REF. [33] WITH PERMISSION)

Compounds	R (%)	CV
Aldicarb sulfoxide	18	24
Aldicarb sulfone	36	18
Oxamyl	70	7
Methomyl	83	2
3-Hydroxy-7-phenolcarbofuran	94	11
De-isopropylatrazine	36	17
Methiocarb sulfoxide	24	21
De-ethylatrazine	60	7
Methiocarb sulfone	47	11
3-Ketocarbofuranphenol	89	6
8-Hydroxybentazone	42	11
Simazine	97	8
Propoxur	91	13
Carbofuran	90	4
Bentazone	81	7
Carbaryl	109	2
Chlorotoluron	100	7
MCPA	34	20
Atrazine	99	4
Isoproturon	100	8
Propanil	94	3
Methiocarb	90	5
Molinate	70	17
Alachlor	90	10

Spiking level, 2.5 μ g/l; water volume, 1 l; in the first extraction 1 g of NaCl was added to the water and the sample was extracted with 50 ml of CH_2Cl_2 . Afterwards the water was acidified with H_2SO_4 at $\text{pH} < 2$ and extracted again. The CH_2Cl_2 fractions were combined and analyzed simultaneously.

ous sample volume up to 980 ml to be extracted with 200 μ l of organic solvent [35]. Although it was applied for the extraction of hydrocarbons, chlorinated pesticides and phthalate esters at trace levels, with average recoveries of 90% by three consecutive extractions, the use of such apparatus is not often described in environmental literature.

Loss or degradation of the analytes can occur during the evaporation step. The analyst is recommended to test the evaporation step by simply spiking the organic solvent directly with the pesticides of interest before evaporation.

4.2.1.4. *Advantages and drawbacks*

The main advantages of LLE are its simplicity and its requirement of simple and inexpensive equipment. Although if it is sometimes difficult to compare recoveries obtained by different laboratories because their extraction conditions (pH, phase ratio, number and time-length of extractions, salinity, etc.) are different, it is easy to modify some of the parameters for a given problem.

An argument often claimed in favour of LLE is that organic contaminants are extracted from both the free water and the suspended matter in a real water. In contrast, only organic compounds dissolved in the water are analyzed using SPE techniques, because surface waters are generally filtered before percolation to avoid plugging of the SPE cartridge, or if they are not prefiltered, filtration will occur within the sorbent bed. It is well known that very hydrophobic organics, such as polychlorinated biphenyls, some organochlorinated pesticides and most of the polyaromatic hydrocarbons, are concentrated more on suspended matter than they are in the dissolved phase [36,37]. Relatively polar pesticides such as atrazine, which exhibits $\log P_{ow}$ values of around 2, are largely distributed in the dissolved -and not in the suspended-phase of the water. This was demonstrated by Pereira and Rostad, with a proportion of 99.5% in water for atrazine, cyanazine, and metolachlor in different surface water samples, indicating that the transport of these pesticides is through the dissolved phase of the river [38]. For moderately polar pesticides, no difference was found between LLE (carried out without filtration) and SPE using a C_{18} filtration disk. Table 4.3 indicates the results obtained with a non-spiked surface water from a river polluted with simazine and atrazine [39]. The concentrations given by the two methods are very close, indicating that these herbicides are in the free water and not in particulate matter.

Further studies are required to assess the true ability of LLE for extracting pesticides from the suspended matter. For solid materials, such as soils and sediments, many studies have demonstrated that when spiking samples in the laboratory, correct recoveries are always obtained. However, this does not indicate that the method can extract the pesticides, because the interactions that occur between the added pesticides and the suspended matter when spiking samples can be very different from those found in natural samples. The widespread practice involves spiking a sample containing suspended matter and humic materials with a mixture of non-polar pesti-

TABLE 4.3

EFFECT OF THE FILTRATION OF SAMPLES OF SURFACE WATERS. CONCENTRATIONS FOUND IN TWO DIFFERENT NON-SPIKED SAMPLES OF SURFACE WATER (USING LLE WITHOUT ANY FILTRATION) AND SPE (FROM REF. [39] WITH PERMISSION)

Sample	Pesticide	LLE ($\mu\text{g/l}$)	SPE ($\mu\text{g/l}$)
1	Simazine	0.29	0.30
1	Atrazine	0.97	1.0
2	Simazine	0.20	0.24
2	Atrazine	0.77	0.92

LLE, extraction from a 500 ml sample, three extractions with 50, 25 and 25 ml of dichloromethane; for SPE, filtration of another 500 ml aliquot of the same surface water sample through an Empore™ C₁₈ disk).

cides dissolved in a 1 or 2 ml aliquot of a water-miscible solvent, mixing it, and allowing it to stand at least overnight prior to LLE. This clearly shows that LLE provides higher recoveries when extracting non polar pesticides in a sample containing suspended matter than does SPE. However, this practice does not demonstrate that LLE is the appropriate method to extract pesticides bound to suspended matter with a 100% recovery. It is known that after 2–3 days contact time, interactions between humic substances and pesticides increase and the recoveries decrease. A better practice is certainly to analyze a non-spiked, but naturally contaminated, surface water sample, to separate both phases, and to measure the amount of pesticides in the suspended matter by different methods (Soxhlet extraction, supercritical fluid extraction, etc.). In a recent study, SPE membrane-disk methods were adapted to allow for the analysis of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in industrial effluents and surface water containing particulate matter [40]. When several types of filtering media were layered on top of the C₁₈ membrane disk, particulate matter and suspended solids could be prevented from plugging the disk, thus allowing an entire sample to be extracted without a need for prefiltering. The analytes of interest, well known for their high hydrophobicity and adsorption properties, could be desorbed directly from the filtering medium and disks using a mixture of toluene and ethanol (30:70, v/v). In these conditions, extensive comparison of LLE and SPE indicated that the two methods were statistically equivalent.

Despite the discussion above, LLE is not free from practical problems such as the formation of emulsions which are sometimes difficult to break. The evaporation of large solvent volumes and the disposal of toxic and often flammable solvents are inherent to the method. The LLE requires several sample-handling steps and contamination and loss have to be avoided at every step. There is a risk of exposure of chemists to toxic solvents or vapours in laboratories. The glassware must be carefully washed, and stored under rigorous conditions. The organic solvents must be very pure and expensive pesticide-grade solvents should always be used when determining traces of pesticides in water.

Carrying out LLE in the field is not easy (except with some continuous extractors) and large water samples are usually transported then stored in laboratories. Automation of the whole procedure of extraction and concentration requires the use of expensive robots, so it is typically an off-line procedure. Loss during transfer and evaporation steps, although small, cannot be avoided. One standard (named a surrogate in EPA methods) is therefore added before LLE and a second one is added to the extract for quantification of the chromatographic analysis. Recoveries should be calculated from the standard peaks by supposing that the losses are similar for the pesticides and standards. Solubilization of the standards in the samples should be assessed carefully. Losses caused by adsorption on vessels are frequently encountered, especially for apolar solutes.

All these above factors explain why liquid-liquid extractions are often described as tedious, time-consuming, costly, and toxic.

4.2.2. Supported liquid membrane (SLM) extraction

Sample preparation by means of liquid membrane extraction is a technique which, in essence, combines two liquid-liquid extractions in one step. It can provide high enrichment factors and a high degree of clean-up, and requires only a few millilitres of organic solvents.

4.2.2.1. Description, set-up and basic parameters

Extraction and enrichment using SLM, connected in a flow system, combines the selectivity and enrichment possible with LLE with the capability of efficient removal of disturbing matrix constituents [7,41]. The SLM techniques involve the use of a porous PTFE membrane separating two aqueous solutions. The membrane is im-

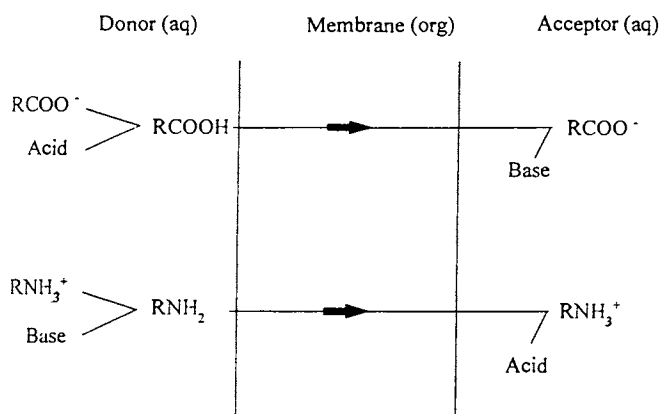


Fig. 4.2. Principles for supported liquid membrane extraction of acidic and basic compounds. From Ref. [7].

pregnated with an organic solvent and mounted between two flat blocks in which grooves are machined, forming a flow channel on each side of the membrane. Other configurations are also possible, e.g., utilizing a hollow fibre impregnated with an organic solvent [8–10]. The device is connected to a flow system, permitting aqueous solutions to be independently pumped through each of the channels. By proper selection of these solutions, compounds can be selectively extracted from one solution (the donor) into the organic membrane liquid and subsequently extracted into the other solution (the acceptor). In Fig. 4.2, we show how the compounds of interest, usually present in ionic form in the donor, together with a suitable reagent, form a non-ionic species which can be extracted into the organic membrane phase. The non-ionic species are then transported through the membrane by diffusing into the aqueous acceptor phase. There, the chemical conditions should be such that the analyte will be converted into a non-extractable form, preventing their re-extraction into the organic phase. In a typical arrangement the acceptor phase is stagnant and can trap a considerable fraction of the analyte of interest which was originally present in the large volume of sample solution pumped through the donor channel.

As an example, the acidic compounds in water are extracted in protonated form from the acidified water sample which is pumped through the donor channel. After passage through the membrane the acids are trapped in a sufficiently alkaline stagnant acceptor phase. In this way, an enrichment factor of several hundreds can easily be attained, with efficient separation from humic substances and other disturbing species in the water samples.

As seen in Fig. 4.2, it is possible to devise a scheme for the extraction of several classes of compounds in a corresponding way, by judicious selection of the pH of the donor and acceptor phases for ionic species. Basic compounds can be extracted from basic donor solutions and trapped in more acidic acceptor solutions. Various charged species can be extracted as uncharged complexes or ion-pairs and trapped on the acceptor side by breaking these complexes in suitable ways.

The selectivity of the extraction process depends primarily on the possibility of transferring the analytes of interest between active and inactive forms in the required sequence, without making the same transfers for interfering compounds. The chemistry of the process is important, and compounds which can be handled by the SLM techniques are mainly ionizable analytes in the pH range 1–14 and compounds which can form complexes.

The SLM technique is in many ways similar to dialysis, and the required equipment is similar. However, in contrast to dialysis where the analytes are diluted, the SLM techniques give an enrichment and a higher selectivity, as dialysis in principle separates small molecules from macromolecules.

4.2.2.2. *Advantages and drawbacks*

The SLM extraction can be used to selectively extract certain classes of compounds while other classes are not extracted. Environmental samples containing high

concentration of matrix constituents such as humic substances and colloidal particles can be processed over long periods. The enrichment is made early in the analytical procedure, which facilitates further operations. The risk of analytical errors is reduced since the influence of contamination and sample loss by adsorption is minimized. The process is performed in a closed flow system, which also minimizes the risk of contamination and can facilitate the handling of dangerous samples. The use of organic solvent is minimal, just a few millilitres to impregnate the membrane. Extraction recoveries can be close to 100% and large enrichment factors can be obtained. In field sampling, several litres can be processed and enriched into a small volume (e.g., 1 ml) of acceptor solution, leading to enrichment factors up to more than 1000. The flow system allows the technique to be easily automated and directly coupled with a subsequent clean-up treatment, if necessary, and with the final analytical chromatographic step. Then, the technique can be integrated with the sampling.

The main drawback is the time of sampling, since percolation of the sample cannot be performed with a high flow rate. One must be careful that degradation of pesticides does not occur during sampling.

4.2.2.3. *Environmental applications*

The SLM technique offers an interesting alternative for selective and efficient extraction of ionic and polar compounds. Applications have been presented for a number of types of analytes in biological and environmental samples. Environmental applications include the extraction of organic acids in manure and soil [42,43], aliphatic amines in ambient air and rain water [44], chlorinated phenols in water [45], the determination of various acidic herbicides such as chlorophenoxy-acid and sulfonylurea herbicides in water [46–50], and the trace enrichment of metals [51]. The SLM technique has been used both off-line and on-line with direct connection to liquid chromatography, using a flow system in which the extracted sample is pumped into the injection loop of the liquid chromatograph [46,49]. A field sampling technique for acidic herbicides has been described where an integrated and specific sampling during 24 h is performed automatically [47,48]. This example is particularly interesting. Continuous sampling from a water stream was achieved for a period of 24 h using a peristaltic pump as shown in Fig. 4.3a, at a flow rate of 0.8 ml/min, and then mixed with a stream of sulfuric acid pumped with a flow rate of 0.15 ml/min. After traversing the membrane, the acidic species, including 2-methyl-4-chlorophenoxyacetic acid (MCPA), were trapped in the 1.5 ml of stagnant acceptor phase (0.1 M phosphate buffer, pH 7) by dissociation, resulting in an enrichment. In such a type of measurement, the extraction efficiency depends on the kinetics of the mass transfer process and not on the equilibrium constant. The conditions can easily be kept sufficiently constant to provide a constant extraction efficiency. Thus, good quantitative accuracy and precision can be obtained. In the experiment described in Fig. 4.3 the extraction efficiency was 0.30–0.34, with a relative standard deviation of 7% using eight replicate measurements with

the same membrane device. The extraction efficiency can be improved by reducing the sample flow rate and can approach unity at a sufficiently low flow rate. However, at higher flow rate, a large amount of analyte can be collected, provided that the available sample volume is sufficient. Figure 4.3b shows the chromatogram corresponding to the analysis of 1 ml of the acceptor solution after an integrated 24 h sampling in a small Swedish brook situated in an area of extensive agricultural activity. Because the acceptor solution is aqueous the final determination was made by reversed-phase LC, utilizing a precolumn instead of a sample loop in the injector. An average concentration of $1.4 \mu\text{g/l}$ of MCPA was measured. Two other phenoxyalkanoic acid herbicides were also identified. This experiment was done during ten consecutive days. Comparisons with a 1 l grab-sampling method and analysis using LLE were made. Large differences have been observed between two grab samples obtained in the same day, depending on the rains. The advantage of such an integrated field sampling was therefore demonstrated if the total leakage of MCPA is to be estimated.

The SLM extraction for the determination of sulfonylurea herbicides is particularly relevant since this is a new class of herbicide used in doses which are substantially lower than for conventional herbicides. This low dose makes determination of the compounds in soils and water difficult, and the sample preparation step must be capable of large, and if possible, selective enrichment. The on-line coupling with LC analysis has been developed [49] for the analysis of four sulfonylureas with detection limits of 50–100 ng/l from the handling of a 250 ml sample.

4.2.2.4. Conclusion

The SLM extraction procedure is a promising technique which will certainly be developed more in the near future owing to its simplicity and its performance. It appears that many pesticides are ionizable in the pH range 1–14. Some of them, which are rather polar, should be amenable to this extraction procedure. However, robustness and validation studies are required in order to implement the technique in routine analysis.

4.2.3. Solid-phase extraction (SPE)

There can be no doubt that solid-phase extraction has now become the method of choice for carrying out simultaneously the extraction and concentration of many pesticides in aqueous samples. Although SPE was introduced more than 15 years ago, and many reviews have reported on its use in environmental analyses [11–13, 16, 52–61], its introduction into official methods, and especially into some EPA methods, has encouraged its development [6]. Owing to the trends for reducing the use of some toxic organic solvents, e.g., chlorinated solvents, we can expect that SPE will be recommended in any future official methods. A recent book dealing with the analysis of micropollutants in water, with its focus on the EU Priority Pollutants List (some 46 pesticides are included in this list), provides clear in-

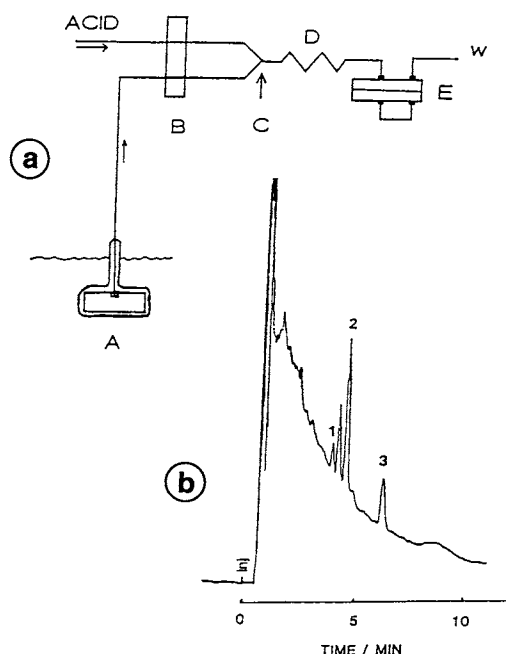


Fig. 4.3. (a) System for integrated field sampling. A, sampling compartment; B, peristaltic pump; C, confluence point of sample stream (0.8 ml/min) and a stream of 0.4 M H₂SO₄ (0.15 ml/min); D, mixing coil; E, membrane separator, with stop flow in the acceptor channel. (b) Chromatogram obtained from an integrated 24 h field sampling of MCPA in a Swedish brook using membrane enrichment. Injection into a precolumn (Hamilton PRP-1 resin). UV detection at 285 nm. Peaks: (1) 2,4-dichlorophenoxyacetic acid (2,4-D); (2) 2-methyl-4-chlorophenoxyacetic acid (MCPA); (3) 2-(2,4-dichlorophenoxy)propionic acid. From Ref. [47] with permission.

structions on the “preferred” sample preparation procedures used in the author’s laboratories, all of which are based on SPE techniques [62]. The availability of cleaner and more reproducible sorbents than in the past has certainly helped its increasing acceptance in the environmental field. Other reasons for the growing interest in SPE techniques are the large choice of sorbents, packed in disposable cartridges or enmeshed in filtration disks, the development of new sorbents which are now capable of trapping the more polar pesticides and/or degradation products, the availability of automatic devices for the whole SPE sequence, and the possibility of the automated monitoring of pesticides on-site by the easy on-line coupling of the SPE process to a chromatographic system. SPE is also still an active area of research,

as shown by the literature and the constant development of new and more selective sorbents.

Although the description of SPE is very simple -i.e., pesticides are trapped by a suitable sorbent, packed in a so-called extraction column through which water passes, and are later recovered by desorption with a small amount of organic solvent-the technique does not appear so simple to any chemist who was not trained in this area. The selection of sorbent, the amount of sorbent, the recoveries which are strongly dependent on the sample volume are not so straightforward. It seems much easier to perform a LLE than a SPE. In fact, SPE is simple when one considers that it isolates compounds in a sample by utilizing the principles of modern liquid chromatography. The SPE sorbents are similar to LC stationary phases and the trapping of the analytes results from the same interactions as involved in LC.

The aim of the following discussion is first to provide the necessary tools for understanding the chemistry behind the SPE process in order to select good experimental conditions for the extraction of any pesticide or metabolite.

4.2.3.1. Description of the off-line methods and automation

SPE can be used off-line, the sample preparation being completely separated from the subsequent chromatographic analysis, or on-line by direct connection to the chromatographic system.

In off-line methodologies, samples are percolated through a sorbent packed in disposable columns or cartridges, or enmeshed in an inert matrix of a membrane-based extraction disk. Disposable prepacked columns or cartridges are available from a number of manufacturers, under various trade names. Containers and reservoirs are generally made of polypropylene. The sorbent bed varies from 100 to 1000 mg and is retained between two porous frits. Volumes above the packing vary from 1 to 70 ml in columns designed with large capacity reservoirs. For larger volume samples, reservoirs can be attached to columns via an adapter or directly to the cartridges. Single samples can be processed by attaching a syringe to the SPE columns or reservoir for application and desorption. The sample can also be aspirated through the column by vacuum. The size of the bed-packing is between 30 and 120 μm so that high flow rates can be applied. Another method of application uses centrifugation, by inserting SPE cartridges into an appropriate centrifuge tube.

A typical SPE sequence involves the steps described in Fig. 4.4 for a cartridge packed with C_{18} silica. First, the SPE column is prepared to receive a sample, by activation or wetting with a convenient solvent, and by conditioning with water (Fig. 4.4a). Then, the aqueous sample is applied, and often the pesticides of interest are trapped together with other components (interferences) of the sample matrix (Fig. 4.4b). Some of these interferences can be removed by application of a washing solution (Fig. 4.4c). This clean-up is examined in more detail later. In the last step, the concentrated analytes are desorbed by applying a small volume of organic solvent which can then be gently evaporated to increase the enrichment factor (Fig. 4.4d).

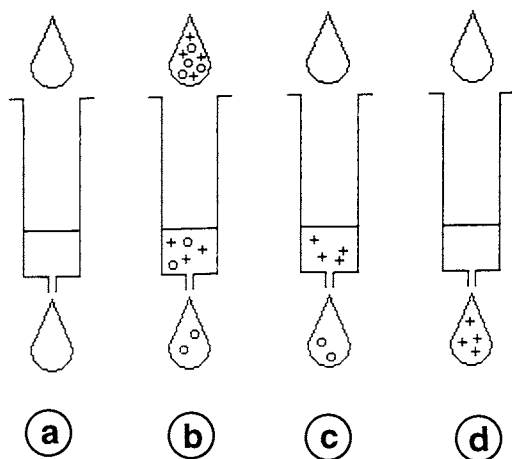


Fig. 4.4. Example of a solid-phase extraction sequence on a disposable column packed with C_{18} silica: (a) conditioning (activation with 3–5 ml of methanol and rinsing with 3–5 ml of deionized water); it is important not to allow the packing to dry out before adding the sample; (b) sample application (+, pesticides and o, interferences); (c) clean-up (washing by applying 1–2 ml of a mixture of water and organic solvent); (d) after drying by vacuum suction for several minutes, desorption occurs in a collecting tube with pure organic solvent, usually 2–5 ml. It is recommended that the solvent wet the sorbent for 1 min and that desorption proceed at a dropwise rate.

The SPE disks have been introduced rather recently [63]. Their main advantages over SPE cartridges is that they allow somewhat higher flow rates without the risk of channelling. The first disks contained the sorbent enmeshed in a Teflon matrix. Recently, new disks have become available with the sorbent in a glass fibre matrix. They are thicker and more rigid and provide faster flow rates than Teflon disks. SPE disks have been tested for various groups of compounds including pesticides, organotins, and phthalates [64–78]. The US EPA has approved various methods based on the use of SPE disks containing either C_{18} silica or a styrene divinylbenzene (SDB) sorbent for the determination of organonitrogen and organophosphorus pesticides (Method 507), of organochloride pesticides and PCBs (Method 508), of acidic herbicides (Methods 515.2 and 555), of paraquat and diquat (Method 549.1), of haloacetic acids and dalapon (Method 552.1) and of benzidines and nitrogen-containing pesticides (method 553) in drinking and source waters. The use of SPE disks is particularly easy. The disks are available with diameter and size similar to liquid chromatographic solvent filters (47 mm and 90 mm). As shown in Fig. 4.5, the membrane is placed in a filtration apparatus attached to a water-aspirator vacuum source, the disk is conditioned with 10 ml of methanol and 10 ml of organic-free water, and the water sample is filtered through it. Then the extraction funnel and frit assembly is transferred to a second vacuum filtration flask containing a test-tube. A

5 ml aliquot of the eluting organic solvent is then drawn through the membrane, with the vacuum being interrupted at this point to allow it to soak the disk for several minutes. This is generally repeated with another 5 ml aliquot. Recently, new apparatus has been developed which gives better performance over the whole procedure. Figure 4.5 shows the Baker Separex Apparatus. All connections are specially designed for carrying out preconcentration studies with the 47 mm disks.

The main advantage of using SPE membrane disks rather than SPE cartridges is the increased productivity permitted by the relatively high flow rates. In general, the time required for the isolation of the various pesticides using disks is half of that using cartridges (30 versus 60 min for 1 l of water). When determining surface or sea water samples, one is recommended to prefilter the samples through 0.45 μm PTFE filters. As the prefiltration can be connected on-line with an EmporeTM disk, the time required for handling water containing suspended matters is much shorter. The use of a 1 μm glass microfibre filter allowed the concentration of 2 l of harbour water in 70 min whereas the EmporeTM disk with no filter required 840 min [70]. An Empore aid filter is available which can be placed on top of extraction disks to a depth of about 1 cm. It is made of glass beads with a typical diameter of 40 μm and is non-porous, inert and inhibits the migration of suspended matter to the surface of the disk. This method also has the advantage of being well adapted to analyzing the partitioning between the dissolved and the suspended phase by analyzing the content of the disk and the glass or PTFE filter, respectively.

Very recently, disks have been introduced in rigid SPE cartridges, known as solid-phase extraction concentrator microcolumns (SPECTM). Their main advantage is the unique rigid disk structure which avoids the creation of voids and channels which can occur in the packed beds of the conventional SPE cartridges. Since there are no frits, the void volume is very small, so the washing and desorption steps may be accomplished very efficiently with small quantities of reagent. However, the amount of sorbent in the available SPEC is 5–56 mg, depending on the diameter and thickness of the disk, which can limit the sample volume and therefore not allow trace analysis at low levels.

Various vacuum manifolds allow batches of up to 24 samples to be prepared simultaneously using cartridges. Special manifolds are also available for the simultaneous extraction of six samples using disks. The application of samples and solvents in the SPE process can thus be performed semi-automatically, with no risk of sample contamination. There is, however, still some manual labour, and the traditional vacuum manifold requires a level of technique to ensure quality data, since all the steps of the SPE sequence need some attention. It is important to watch columns or disks to prevent their going dry before sample application, and to take care of the desorption step.

The sequence can be totally automated (ASPEC system from Gilson or Auto Trace SPE Workstation from Zymark). These devices allow consistent processing of the samples through automation and eliminate operator variability, since they incorporate microprocessor flow control of samples.

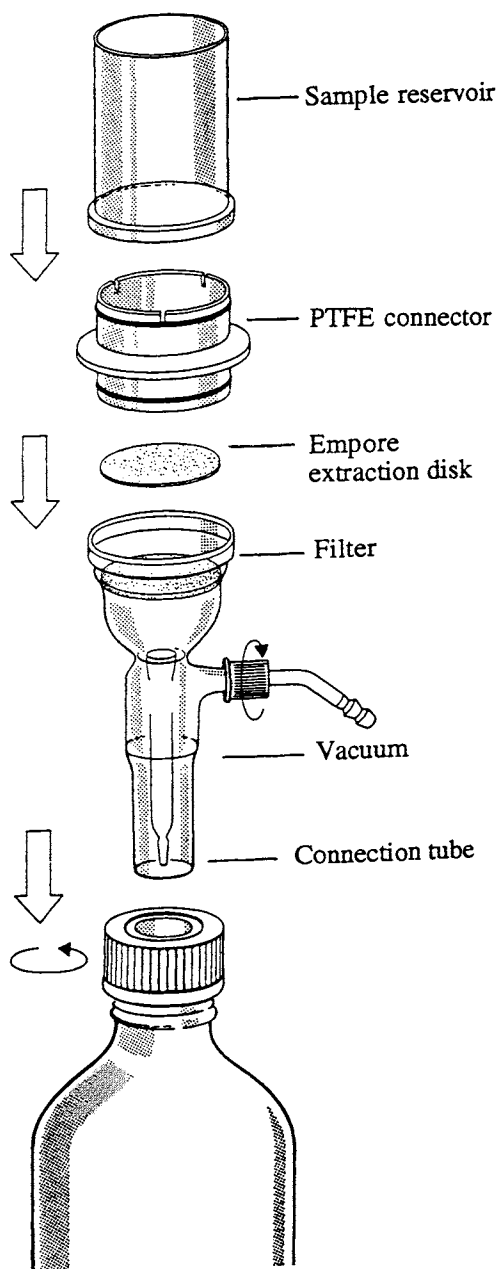


Fig. 4.5. Baker Separex extraction apparatus. From Ref. [72].

Compared with LLE-based sample preparation, off-line SPE offers reduced processing times and substantial solvent savings. As discussed in detail in Chapter 2, percolation of samples can easily be performed in the field, and good storage of ad-

sorbed analytes has generally been observed [16,53,72–74,77]. The problem of transport and storage of voluminous samples is avoided, which is especially interesting when samples have to be taken from remote sites. Automation is possible using robotics or special sample-preparation units that sequentially extract samples and clean them up for automatic injections. Nevertheless, a certain amount of tedious labour remains and off-line procedures have the inherent disadvantages of loss in sensitivity owing to the injection of an aliquot, losses in the evaporation step, and some risks of contamination, so internal standards are required. On-line coupling of the SPE sample preparation to a GC or LC separation avoids many of these problems, because the entire sample is transferred and analyzed, which allows the handling of smaller volumes.

4.2.3.2. Basic principles

The chemistry and principles are essentially identical for both off-line, using disks or cartridges, and on-line SPE using small precolumns. The processes involved in SPE are a frontal chromatography during the extraction step and a displacement chromatography during the desorption step. These two modes are well known and, to a first approximation, SPE can be described as a very simple chromatographic process, the sorbent being the stationary phase. The mobile phase is the water of the aqueous sample during the extraction step or the organic solvent during the desorption step. Retention of organic compounds occurs to the extent that they are not eluted by water during the extraction step. High enrichment factors are obtained when the analytes are strongly retained by the sorbent in the presence of water and when there is low retention with the organic solvent used for the desorption.

The choice of the sorbent is guided first by the aqueous nature of the samples. Water should not elute the target pesticides. Thus, bare silica, and silica modified with polar groups, are not usually appropriate sorbents because water is the strongest eluting mobile phase. Better sorbents are the reversed-phase stationary phases for neutral pesticides (mainly alkyl-modified silicas, apolar copolymers and carbonaceous sorbents) and ion-exchangers for ionized compounds.

4.2.3.2.1. Breakthrough volume. Breakthrough occurs either when pesticides are no longer retained by the sorbent or when the capacity of the sorbent has been overloaded. The latter situation is unlikely to occur in practical environmental analysis of pesticides where the concentrations are typically of the order of $\mu\text{g/l}$. The breakthrough is mostly caused by insufficient retention.

Breakthrough volumes can be measured by monitoring the UV signal of a water sample spiked with traces of a pesticide, S, which has an initial UV absorbance A_0 as shown in Fig. 4.6a. The spiked sample is percolated through a SPE column. If the compound is retained by the sorbent, the effluent will not contain it, and its UV absorbance will be zero. A frontal or breakthrough curve is recorded, beginning at a volume, V_b , usually defined as 1% of the initial absorbance A_0 up to a volume, V_m ,

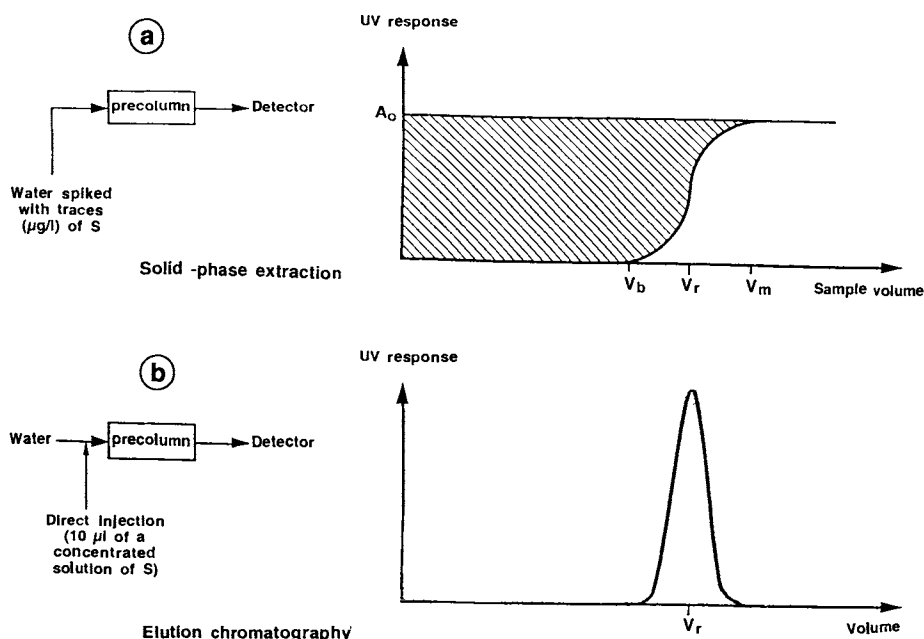


Fig. 4.6. Similarity between SPE and elution chromatography. From Ref. [55] with permission. (a) Breakthrough curve obtained by recording the UV signal of the effluent from a precolumn (or cartridge) when percolating a water sample spiked with a solute S. The absorbance of the spiked solution is A_0 and was measured by direct connection of this solution to the UV detector. The breakthrough volume is usually defined as 1% and V_m at 99% of the initial absorbance. V_r is measured at the inflection point of the curve. The shaded area shows the maximum amount that can be pre-concentrated. (b) Elution peak obtained by injecting 10 μl of a concentrated solution of S in the precolumn eluted with a mobile phase of water only. The flow rate is the same as in (a), and the retention volume of the peak is V_r .

defined as 99% of the initial absorbance, where the effluent has the same composition as that of the spiked water sample (Fig. 4.6a). Under ideal conditions, this curve has a bi-logarithmic shape, the inflection point of which is the retention volume, V_r , of the analyte. When using the same SPE column in elution chromatography with water as the mobile phase and with the same flow rate, the injection of 10 or 20 μl of a concentrated solution of the same pesticide S will generate a peak detected at the same volume V_r , as represented in Fig. 4.6b.

The quantity V_b is a key parameter for the pre-concentration of the analytes and can be estimated to a first approximation from V_r [53,79–82]. The similarities between SPE and LC indicate that the data generated by LC for measuring or estimating V_r are useful since predictions of V_r can thus be made. Knowledge of the retention behaviour of analytes with hydrophobic sorbents should be applied. For example, ionizable compounds are only retained on C_{18} silica in their neutral form, so the

pH of the water sample is an important parameter, especially for the extraction of the weakly acidic or basic pesticides. In practice, one first needs an approximate value of V_b in order to select an appropriate sorbent and the amount of sorbent for off-line preconcentration with cartridges.

4.2.3.2.2. Recovery. Recovery is defined as the ratio of the amount extracted to the amount percolated, and is theoretically 100% only for a sample volume equal to or lower than V_b . The maximum amount preconcentrated is reached for a sample volume equal to V_m (hatched area in Fig. 4.6a) and does not correspond to a 100% recovery. Therefore, the recovery in SPE depends on the sample volume percolated and on the breakthrough volumes, which are related to the amount and nature of the sorbent.

Recoveries obtained with the same sorbent from one experiment can be compared with another only if the amount of sorbent and the sample volume are known. It is always possible to obtain a 100% recovery by reducing the sample volume below the corresponding V_b , and a simple calculation indicates whether this volume will allow the required level of detection or not.

4.2.3.2.3. Limit of detection and breakthrough volume. The absolute limits of detection (LOD) of the chromatographic detection system are usually expressed in μg or ng injected (e.g., 10 ng). The LOD are experimentally measured once the separation has been optimized by loop injection and are defined as a signal-to-noise ratio of 3. Therefore, it is easy to calculate the theoretical detection limit expressed in concentration units in the aqueous sample, C_{lim} , which corresponds to the maximum amount that can be preconcentrated (hatched area in Fig. 4.6a) or as equal to the product $C_{\text{lim}} \times V_r$. If V_r is 100 ml and the LOD is 10 ng, for example, then the concentration limit is 0.1 $\mu\text{g/l}$. This value has been calculated on the basis that the whole extract is injected in the chromatograph, which never occurs in off-line methods. If the extract is obtained in 500 μl and only 100 μl are injected, then C_{lim} is 0.5 $\mu\text{g/l}$. If a lower C_{lim} value is required, the only remedy is to increase V_b (or V_r) by increasing the amount of sorbent or by selecting another sorbent that will provide a higher retention in the presence of water for the pesticides of interest. This calculation does not take into account the effect of the sample matrix, which can dramatically increase the C_{lim} , because of interfering compounds. The limit of quantification (LOQ) is usually defined as five to ten times the LOD expressed in concentration and measured with the sample matrix. In other words, a LOD of 10–20 ng/l should be required in drinking water samples to enable quantification at the 0.1 $\mu\text{g/l}$ level.

4.2.3.2.4. Experimental determination of breakthrough volumes and recoveries. Recording breakthrough curves is time consuming and reading V_b at the 1% level is neither easy nor always accurate. The sample should be spiked at a trace level in order not to overload the sorbent capacity, and the UV signal should be monitored at

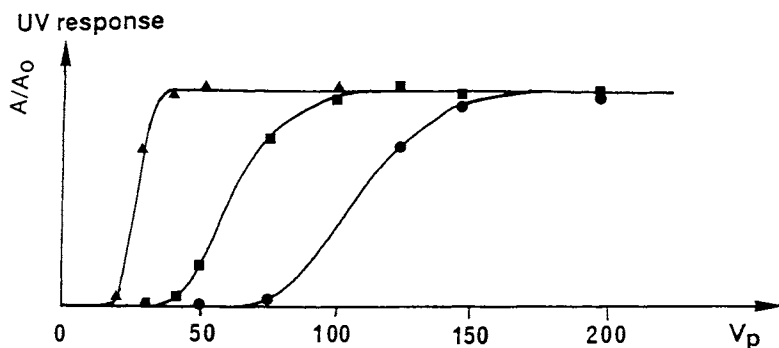


Fig. 4.7. Experimental breakthrough curves recorded with a 1 cm x 0.21 cm i.d. precolumn packed with RP-18 silica. Samples: solution spiked with 100 $\mu\text{g/l}$ of (Δ) simazine, (\blacksquare) atrazine and (\bullet) linuron. From Ref. [79].

very low absorbances, which may lead to problems with baseline stability or noise. Moreover, some pesticides are difficult, and sometimes impossible, to record because they have poor UV properties. Figure 4.7 shows experimental breakthrough curves obtained for three herbicides with a 10×2.1 mm i.d. precolumn packed with C_{18} silica. These curves are different, and the more retained the compound is, the larger volume the curve is spread over, because of the low plate number of the precolumn. The front corresponding to linuron spreads over nearly 100 ml from a V_b value of 70 ml to a V_m value of 165 ml. First, the determination of V_b at 1% of the initial absorbance on the front curves cannot be accurate when the front is not sharp. The second point is that if no breakthrough is wanted for a 100% recovery, the percolated volume has to be lower than 70 ml. Nevertheless, raising the percolated volume to 165 ml considerably increases the amount preconcentrated by nearly to 50%. The corresponding recovery is then below 100%, but overcoming the breakthrough volume may sometimes be interesting when traces of organic compounds have to be determined in water samples having relatively low organic contamination. Of course, the same situation occurs for some of the analytes when many solutes of different polarity are to be determined together.

A faster method for estimating breakthrough volumes and recoveries has been developed [79,82]. It is easily performed with the on-line apparatus, but can also be carried out using off-line preconcentration. It consists of preconcentrating water samples of increasing volumes, each containing the same amount of analytes, and then measuring the peak-areas or heights eluted on-line from a precolumn, or off-line from a cartridge or disk. As the sample volume increases, the analyte concentration decreases, provided breakthrough does not occur: the amount preconcentrated remains constant and the peak areas in the on-line chromatograms following desorption are constant. When breakthrough occurs, the amount extracted is reduced, and the desorption peak-area or height decreases. The corresponding recoveries can be

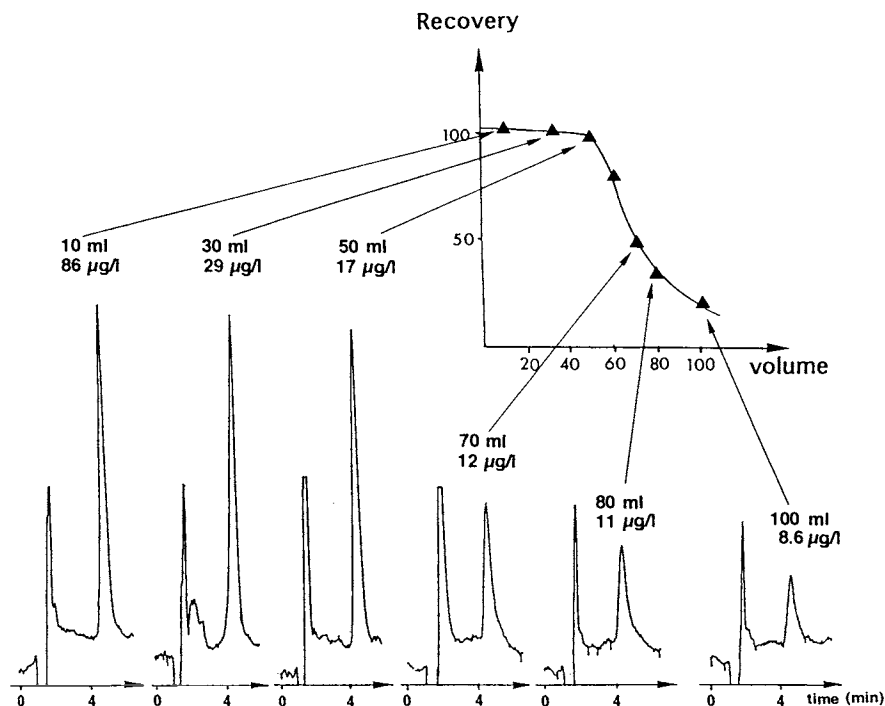


Fig. 4.8. Experimental determination of the breakthrough volume and corresponding recovery. From Ref. [55] with permission. Different sample volumes, containing the same amount of cyanuric acid ($0.86 \mu\text{g}$), are percolated through a $1 \times 0.46 \text{ cm}$ i.d. precolumn packed with porous graphitic carbon, PGC ($10 \mu\text{m}$). The chromatograms correspond to the on-line elution of each sample using a $10 \times 0.46 \text{ cm}$ i.d. analytical column prepacked with PGC (Hypercarb) using a mobile phase containing 30% methanol and 70% 0.05 M sodium phosphate at pH 7; flow rate 1 ml/min; UV detection at 220 nm. Recoveries are calculated from the ratio of peak areas. The sample volume and the corresponding concentration are indicated on each chromatogram.

calculated by dividing the peak areas obtained after breakthrough by those obtained before. This is shown in Fig. 4.8. An advantage of this method is that the V_b values of several compounds can be estimated simultaneously by preconcentration and analysis under the real experimental conditions of unknown samples, via the whole off-line or on-line procedure.

4.2.3.2.5. Prediction of breakthrough volumes and recoveries from LC data. The breakthrough volume can be estimated using V_r , which is related to chromatographic data and cartridge or precolumn characteristics, using the relationship

$$V_r = V_0(1 + k'_w) \quad (1)$$

where V_0 is the void volume of the precolumn or the cartridge and k'_w is the retention factor of the solute eluted by water, defined by $k'_w = (V_r - V_0)/V_0$. The void volume can be calculated from the porosity of the sorbent (ϵ) and the geometric volume (V_c) of the precolumn or sorbent bed in the cartridge or disk ($V_0 = \epsilon \times V_c$). Most of the reverse phase sorbents used in cartridges have an average porosity between 0.65 and 0.70. Cartridges are often characterized by an amount of sorbent. With an average density of 0.6 g/ml for the C_{18} silica used in cartridges, V_0 can be estimated as 0.12 ml per 100 mg of sorbent.

Values of k'_w are often estimated from chromatographic measurements using analytical columns packed with reversed phase sorbents, such as C_{18} silicas which are eluted with a mobile phase composed of water–methanol mixtures. The advantage of this method is that experimental data are obtained rapidly by measuring the retention factor k' of the analyte in methanol–water phases. Over a range of methanol concentration, often between 15% and 90%, there is a linear relationship between $\log k'$ and the percentage of methanol. As shown in Fig. 4.9a., this has been observed for phenol using alkyl silicas, apolar SDB copolymers PRP-1 (from Hamilton), and porous graphitic carbon, PGC (from Shandon).

From rapid measurements with three or four mobile phases containing different

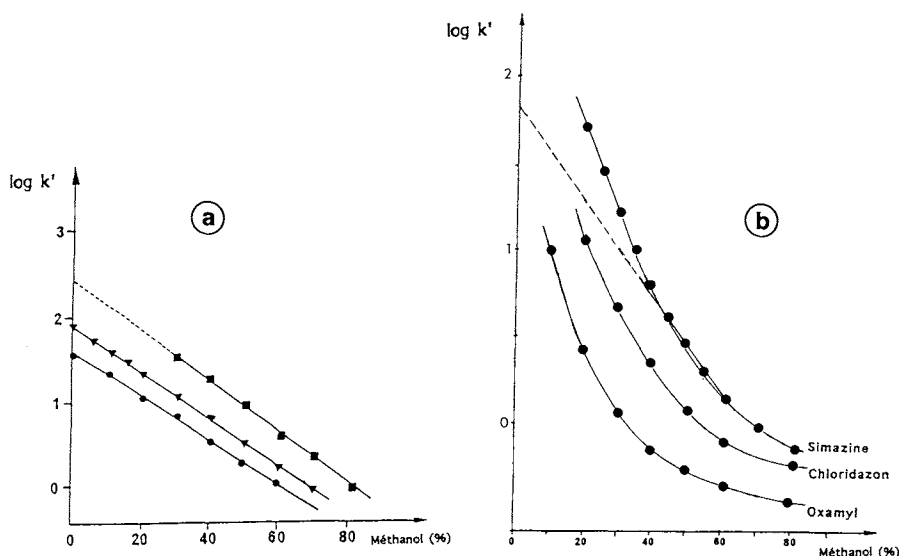


Fig. 4.9. (a) Variation of the retention factor of phenol with the percentage of methanol in the water–methanol mobile phase as measured with (●) C_{18} silica RP-18 (from Merck), (■) PRP-1 SDB copolymer (from Hamilton) and (▼) Hypercarb PGC (from Shandon). From Ref. [55] with permission. (b) Variation of the retention factor of various pesticides with the percentage of methanol in the water–methanol mobile phase as measured with C_{18} silica, RP-18, using a 5 cm long column to obtain experimental data in water-enriched mobile phases. From Ref. [85].

methanol concentrations, k'_w can be estimated by graphically extrapolating to zero-methanol content. In Fig. 4.9a the experimental value of $\log k'_w$ should be similar to the predicted value because the relationship is linear over the entire range. Werkhoven-Goewie et al. [80] found good agreement between the V_r values for some chlorophenols, derived from experimental breakthrough curves, and the values calculated from extrapolated k'_w values. Differences were of the order of 10–20%. However, according to Schoenmakers et al. [83], the relationship between $\log k'$ and the methanol content is a better fit with a quadratic relationship for some compounds. Values extrapolated from a linear part of the curve can be lower, similar, or higher than the experimental ones depending on the solutes. Jandera and Kubat [84] have shown that, when using a short microcolumn (30×1 mm i.d.), for some solutes it was possible to measure k'_w directly by elution methods, and that for the more apolar herbicides the extrapolations are more rapid and more accurate because the experimental range for extrapolation is water-rich. This is clearly illustrated in Fig. 4.9b, where a very short analytical column has been used in order to obtain experimental data in water-enriched mobile phases. When the k' values of simazine are only measured in the range 60–45% methanol, the linearly extrapolated value will be lower than 2 whereas this value is close to 3, using a quadratic extrapolation and experimental data in mobile phases containing a high proportion of water. From these curves, we can see that the extrapolated values from quadratic relationships are more appropriate for estimating $\log k'_w$, and that only an approximate value can be derived from these curves: however, for trace enrichment studies, this approximation gives a good estimation of the breakthrough volumes and recoveries. As an example, the $\log k'_w$ value of cyanuric acid was estimated as 2.5 ± 0.2 using the extrapolation method, which gave a V_r value between 30 and 70 ml, for the precolumn used in Fig. 4.8 (assuming a porosity of 0.75 for the porous graphitic carbon). The experimental V_b was measured as 50 ± 5 ml in Fig. 4.8.

The V_b values can be also calculated from the k'_w values as developed below, because V_r is linked to V_b as defined in Fig. 4.6. by the relationship (when V_b is read at 1% of the initial absorbance)

$$V_b = V_r - 2.3\sigma_v \quad (2)$$

where σ_v is the standard deviation depending on the axial dispersion along the bed of particles in the precolumn or cartridge. The breakthrough volume is therefore controlled by retention and kinetic parameters [16,53,55,86,87]. As seen above, the V_r term can be calculated from k'_w and V_0 (see Eq. (1)). The σ_v term can be calculated if the number of theoretical plates, N , of the precolumn or cartridges is known:

$$\sigma_v = (V_0/\sqrt{N})(1 + k'_w) \quad (3)$$

It is only possible to measure N directly with precolumns because the on-line system

can allow recording of the breakthrough curve and the elution peaks by direct injection onto a precolumn [79]. Because the precolumns are packed with stationary phases similar to those used in LC, an estimation of N can also be derived from the average number of plates in an analytical column. It is much more difficult to measure the efficiency of a SPE cartridge or that of an extraction disk, so N has to be estimated. SPE cartridges are very different from LC columns and the average particle size is much larger, so that a poor efficiency is expected. Miller and Poole [86] have recently studied the kinetic and retention properties of an SPE cartridge packed with 500 mg of C_{18} silica and they measured an average of 20 theoretical plates for a flow rate of 5 ml/min.

The breakthrough curves, as described in Fig. 4.6, have been modelled and the effect of $\log k'_w$ on the shape of the curves has been studied [85] for a sorbent having a void volume of 0.54 ml (which corresponds to an extraction disk containing 450 mg of sorbent) and with 20 plates. Figure 4.10a shows the general shape of the breakthrough curves depending on the $\log k'_w$ values of the analytes, varying in the range 1.5–3.9 which corresponds to a range of relatively polar to non-polar pesticides. The corresponding theoretical recovery curves are represented in Fig. 4.10b. First, the more polar the analytes are, the sharper the fronts are. Breakthrough occurs for a volume lower than 500 ml for all the compounds with $\log k'_w$ values lower than 3.3. However, even if breakthrough occurs the effect on recoveries is not so important. An examination of the corresponding recovery curve is relevant. If we look at a compound characterized by a $\log k'_w$ of 2.9 (calculated V_r value of 430 ml), although breakthrough has occurred for a calculated volume of 210 ml, the theoretical recovery value obtained with a sample volume of 500 ml is still around 85%. Only compounds with $\log k'_w$ lower than 2.5 will be extracted with recoveries lower than 50% with a 500 ml sample volume. A volume of 500 ml has been selected because this is a typical sample volume for off-line extraction allowing multiresidue determination at a low 0.1 $\mu\text{g/l}$ level for many pesticides in drinking water samples. As already pointed out, both the breakthrough and the recovery curves also show the difficulty in determining the corresponding breakthrough volumes with accuracy for an analyte having a $\log k'_w$ value above 2.5. We can also conclude from these curves that knowledge or an estimate of the retention factor in water, k'_w , is the most relevant parameter to be known for predicting whether or not a compound will be extracted by a sorbent. Some recent studies have also pointed out the role of the retention factor in method development for SPE [88–93].

The recovery depends on the amount of sorbent. These curves have been calculated for 450 mg of sorbent and will be just slightly different for the widely used cartridges packed with 500 mg of sorbents. According to Eq. (1), V_r is directly proportional to V_0 and therefore to the amount of sorbent. The retention factors do not depend on the amount of sorbent, but only on the nature of the sorbent. In conclusion, using a 500 mg cartridge or a 47 mm disks only pesticides and degradation products characterized by $\log k'_w$ values lower than 3 will lead to incomplete recov-

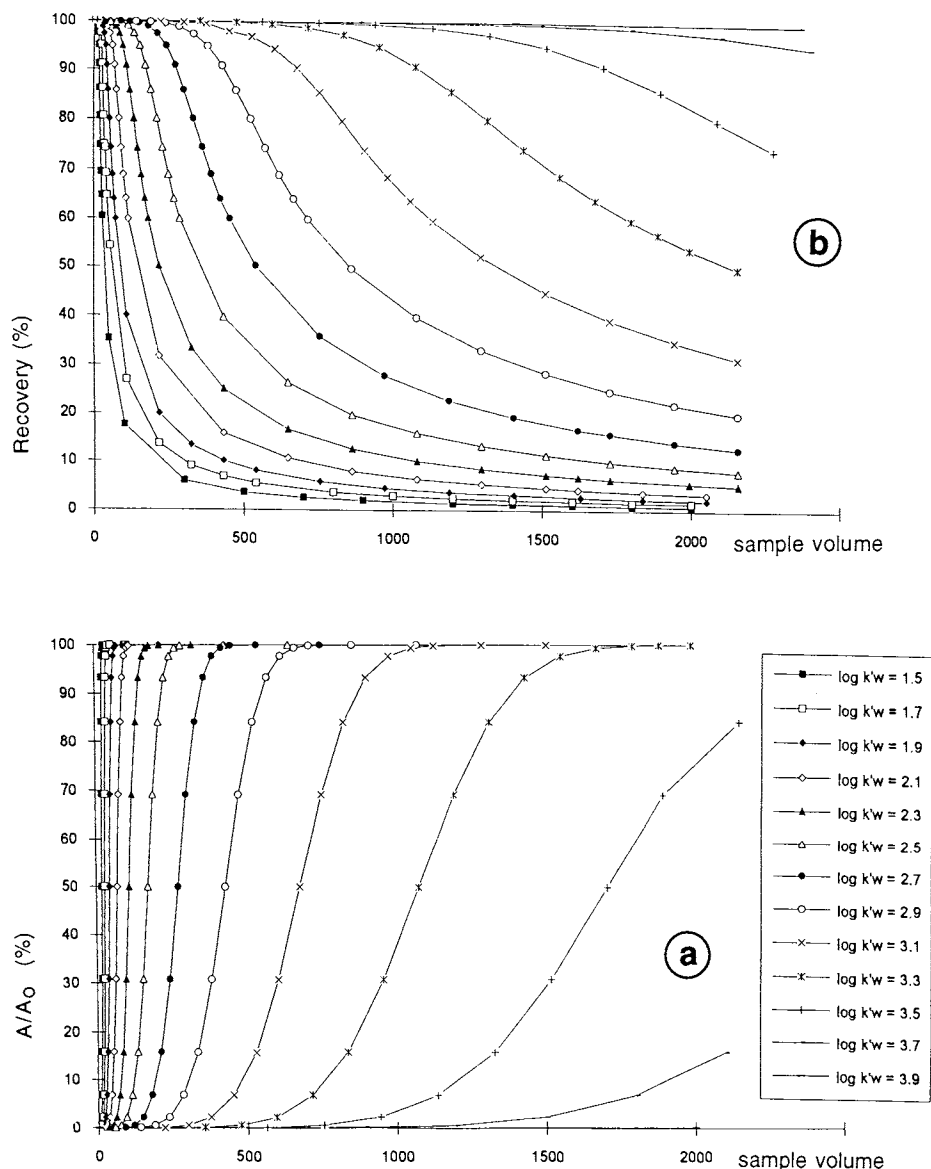


Fig. 4.10. Effect of the $\log k'_w$ values of the analyte on (a) theoretical breakthrough curves and (b) the corresponding theoretical recovery curves assuming 20 plates in the cartridge or disk.

eries if a 500 ml sample is required. If a sample of 100 ml is sufficient, then only compounds with $\log k'_w$ lower than 2.5 will not be totally recovered.

The agreement between experimental and theoretical recovery curves has been obtained for a set of polar pesticides, using two types of extraction disks containing 450 mg of C_{18} silica and 450 mg of styrene divinylbenzene polymer, respectively.

TABLE 4.4
COMPARISON BETWEEN EXPERIMENTAL AND PREDICTED BREAKTHROUGH VOLUMES

Pesticide	C ₁₈ disk			SDB disk		
	log k'_w	Predicted V_b	Experimental V_b	log k'_w	Predicted V_b	Experimental V_b
Oxamyl	1.7 ± 0.1	14 ± 4	25 ± 5	2.8 ± 0.2	170 ± 80	250 ± 50
De-isopropyl-atrazine	2.3 ± 0.1	60 ± 15	80 ± 10	3.2 ± 0.2	475 ± 200	650 ± 50
De-ethylatrazine	2.7 ± 0.1	130 ± 45	220 ± 20	3.5 ± 0.2	840 ± 400	1000 ± 100
Aldicarb	2.5 ± 0.1	84 ± 20	80 ± 10	4.0 ± 0.2	>1600	>1500
Simazine	3.4 ± 0.2	670 ± 150	>500	4.1 ± 0.2	>2100	>1500

Extrapolated log k'_w values obtained from experimental k' measurements using columns packed with the C₁₈ and SDB sorbents, calculated V_b values in ml, and experimental values in ml, determined from preconcentration of spiked solutions using C₁₈ and SDB disks (47 mm diameter, from J.T. Baker).

The log k'_w values were extrapolated from values of k' measured in a methanol–water mixture according to the method described in Fig. 4.9b and by using a 5 cm long × 0.46 cm i.d. column packed by piling up small disks of stationary phases. The results are reported in Table 4.4 for C₁₈ and SDB disks. Then the V_b values were calculated according to Eqs. (1)–(3). Experimental recovery curves, depending on the sample volumes, have been measured by spiking solutions of LC-grade water with a constant amount (40 µg) of each pesticide and increasing the sample volume from 10 and 1500 ml. The agreement between calculated and experimental values reported in Table 4.4. is good if one takes account of the difficulty mentioned above for the accurate determination of V_b values from the curves reported in Fig. 4.10b. Figure 4.11 reports the experimental variations of the recoveries with the sample volume, and the calculated curves for oxamyl and deethylatrazine using a C₁₈ and an SDB disk, respectively. Taking account of the fact that recoveries are obtained with an average error of 10% as a consequence of the different steps of the sequence, the agreement between calculated and experimental curves is very good. Thurman et al. have measured values of volumes at 10% of breakthrough of 75 ml for de-isopropyl-atrazine, and 300 ml for de-ethylatrazine, using a 350-mg C₁₈ cartridge [94]. One should also mention the great difference in V_b values obtained using a C₁₈ disk and an SDB disk. The advantage of using a sorbent providing a larger retention factor in water is shown, since for oxamyl a recovery of 25% is observed for a 100 ml sample with a C₁₈ disk and for a 1000 ml sample with an SDB disk.

Calculations of V_b are rather time-consuming and their accurate experimental determination is difficult. Because, in practice, we just need a rapid approximation of V_b to help us to select the sorbent we have also looked at the approximation of V_b by V_r values, which are easily calculated from V_0 and log k'_w values. In Table 4.5, we report V_r and V_b values, and the recoveries which are obtained for V_r values. For low

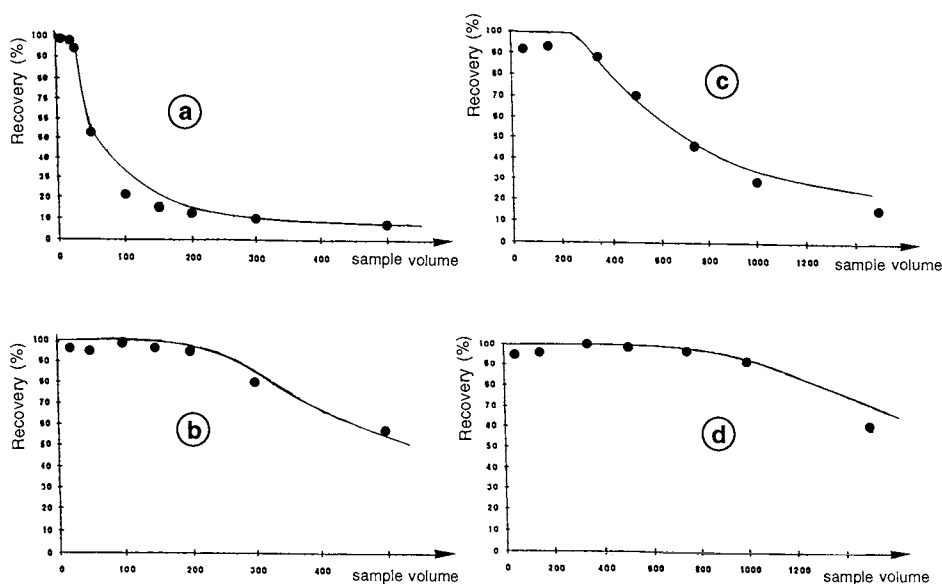


Fig. 4.11. Predicted recovery curves (plain line) obtained for oxamyl and de-ethylatrazine (DEA) with the sample volume and (●) experimental values. (a) Oxamyl and (b) DEA using a C₁₈ Empore™ disk from J.T. Baker, diameter 47 mm, LC-grade water sample packed with a constant amount (40 μg) of analyte. Sequence of extraction: disk preconditioned with 10 ml of a 50:50 (v/v) acetonitrile–methanol mixture, then with 10 ml of methanol and equilibrated with 20 ml of LC-grade water, sample applied at a flow rate of 25 ml/min, air drying and desorption with 2 × 4 ml of 50:50 (v/v) acetonitrile–methanol mixture at low flow rate, evaporation of the solvent mixture, and injection into a LC system. (c) Oxamyl and (d) DEA, using an SDB Empore™ disk from J.T. Baker, diameter 47 mm, LC-grade water sample packed with a constant amount (40 μg) of analyte. Same sequence of extraction, except preconditioning with 10 ml of acetone and desorption with 2 × 6 ml of the methanol–acetonitrile mixture.

values of $\log k'_w$ (<2.1) the difference between V_r and V_b is not large because the front-shape is sharp. The difference becomes important for $\log k'_w$ above 2.5 because the breakthrough curves spread (V_r value calculated as 170 ml, V_b calculated as 84 ml and the experimental V_b is observed at 80 ± 10 ml). For higher $\log k'_w$ values, the fronts spread over more than 200 ml and the approximation is no longer valid. However, Table 4.5 shows that when samples of volume V_r of all these compounds are percolated, the corresponding recoveries are above 70%.

4.2.3.3. Selection of the sorbents and desorption conditions, and effect of the sample matrix

The choice of the sorbent depends on the volume to be handled for the required detection and on the nature of the pesticide of interest. As shown in the previous section, a good knowledge of the retention behaviour of pesticides with the main LC

sorbents is of great help in the selection of the sorbent. The analyte extraction depends on the various interactions which will be discussed separately for each type of sorbent in this section. The conditions for desorption also depend on the retention mechanism involved with the sorbents, and will be examined for each one. Non-selective sorbents, i.e., reversed-phase sorbents, are examined first. Some selective sorbents, such as ion exchangers, metal-loaded sorbents, and the new immuno-affinity sorbents are also discussed.

The characteristics of each sorbent are illustrated by various applications with real samples, showing the effect of the sample matrix and the detection limits which can be obtained in environmental water.

4.2.3.3.1. *N-Alkyl silicas.* Most of the published off-line procedures now use disposable cartridges or filtration disks packed with C_8 or C_{18} silicas. Alkyl silicas are very pressure-resistant and are available in various granulometries, typically from 3 to 200 μm . Their main drawback is their poor stability in very acidic or basic media, which limits their use to the pH range 2–8. Nevertheless, their good reproducibility in retention, rapid equilibrium with mobile phases, and the rarity of irreversible adsorption of analytes, explain their widespread use in LC and in extraction procedures.

Prediction from hydrophobicity parameters and limitation for polar pesticides. It is important to know which pesticides can be preconcentrated from a sufficient volume for trace analysis using C_{18} sorbents. In reversed-phase chromatography it is well known that hydrophobic compounds are well retained with water as the mobile phase, and that their separation requires a mobile phase containing an organic solvent, whereas polar analytes are difficult to separate with an aqueous mobile phase. The limitation on using an *n*-alkyl silica will therefore occur for the more polar pesticides and degradation products. Because of the fact that C_{18} silicas usually provide higher retention than C_8 silicas when the silica surface is totally covered by alkyl chains, C_{18} silicas should be used for extracting polar pesticides.

Cartridges available are packed with C_{18} silicas having a variety of characteristics,

TABLE 4.5
COMPARISON BETWEEN RETENTION (V_r) AND BREAKTHROUGH (V_b) VOLUMES AND RECOVERIES OBTAINED WHEN PERCOLATING SAMPLE VOLUMES EQUAL TO V_r

Pesticide	C_{18} disk			
	$\log k'_w$	Calculated V_r (ml)	Predicted V_b (ml)	Recovery at V_r (%)
Oxamyl	1.7 ± 0.1	28 ± 5	14 ± 4	65–75
De-isopropylatrazine	2.3 ± 0.1	108 ± 20	60 ± 15	70–80
Aldicarb	2.5 ± 0.1	170 ± 45	84 ± 20	>80
Deethylatrazine	2.7 ± 0.1	270 ± 70	130 ± 30	>85

and it is well established that in LC the retention differs from one C_{18} stationary phase to another, because the retention depends on the number of C_{18} chains bonded at the surface of the silica [95]. Nevertheless, except for C_{18} silicas with a low surface coverage of C_{18} moieties (with a low carbon content), k'_w is comparable (within a 20% variation) for most C_{18} silicas, which is still acceptable for prediction. We have recently verified the 20% variation by using several commercial C_{18} columns and by packing columns with C_{18} sorbents from several C_{18} cartridges [85]. Higher k'_w values were obtained with cartridges of sorbents which had been synthesized from silicas having large specific areas. Several C_{18} SPE cartridges specifically designed for trapping polar analytes are now available. In LC, in order to obtain a better efficiency and a totally apolar material, the trend is to minimize the number of residual silanol groups of the original silica: for this purpose, a trifunctional silane is used for bonding the *n*-alkyl chains and an "end-capping" is carried out with trimethylsilane after bonding. However, the purpose of an extraction is different from LC separation, and it was observed that the contact between some polar analytes and a totally hydrophobic C_{18} silica during the SPE process was better when the C_{18} silica was not (or was only slightly) end-capped, or contained some polar groups in addition to the alkyl chains [96]. The use of a monofunctional silane without end-capping provides the highest amount of residual silanol groups. A consequence is that secondary interactions, such as hydrogen bonding, can occur between silanol groups and polar pesticides, thus facilitating their retention [97]. Stronger secondary interactions can also occur with basic analytes [98]. These specific C_{18} silicas for the extraction of polar pesticides should not be confused with "light loaded C_{18} silicas", which are characterized by a low carbon content. Standard C_{18} sorbents and those specifically designed for polar analytes generally have similar carbon contents, so that their retention of non-polar analytes is similar, whereas a lower retention is observed with those having a lower carbon content.

Since the retention mechanism is primarily governed by hydrophobic interactions between the analyte and the carbonaceous moieties of the alkyl chains grafted at the silica surface, a relation has been observed between the retention factors of the analytes and their water-octanol partition coefficient (P_{ow}). The latter characterizes well the hydrophobicity of a compound and plays an important role in correlating the phenomena of physical, chemical, biological, and environmental interest [99–101] as seen in the first chapter. Braumann [100] has gathered many $\log k'_w$ values obtained with different C_{18} silicas using methanol–water as the mobile phase. A linear relationship was found between the average $\log k'_w$ values and $\log P_{ow}$ for closely related compounds and even for compounds having different polarities and chemical properties. For example, 60 compounds covering a wide range of structures, from polar aniline ($\log P_{ow} = 0.91$) to the very hydrophobic *p,p'*-DDT ($\log P_{ow} = 6.2$), are related by $\log k'_w = 0.988 (\pm 0.051) \log P_{ow} + 0.020 (\pm 0.060)$. Therefore, k'_w values can be approximated without any additional measurements when $\log P_{ow}$ values are available. Chlorophenols were used as an example: depending on the number of sub-

TABLE 4.6

RELATION BETWEEN THE WATER–OCTANOL PARTITION COEFFICIENT AND THE RETENTION FACTOR IN WATER

Solute	$\log P_{ow}$	$\log k'_w$	Retention volume (ml)
Pentachlorophenol	5	4.8	7350
2,4,5-Trichlorophenol	4.1	4.0	1064
3,5-Dichlorophenol	3.6	3.5	370
2,4-Dichlorophenol	3.2	3.1	160
4-Chlorophenol	2.4	2.3	32
2-Chlorophenol	2.2	2.1	16
Phenol	1.5	1.6	5

Approximate retention volumes, V_r , calculated for SPE cartridges packed with 100 mg of C_{18} silica from the water–octanol partition coefficient ($\log P_{ow}$) or from the extrapolated retention in water ($\log k'_w$). From Ref. [55] with permission.

stituents, this series ranges from apolar ($\log P_{ow} = 5$) to rather polar compounds ($\log P_{ow} = 1.5$). In Table 4.6 are values of $\log k'_w$ obtained by extrapolation from the change in k' with methanol content; these are in good agreement with the literature [100]. One can therefore rapidly obtain V_r from $\log P_{ow}$ values and determine whether, depending on the concentration limit required, C_{18} silica is suitable for the extraction. The data reveal significant differences between apolar and moderately polar compounds: for 100 mg of C_{18} silica the V_b values are 7 l for pentachlorophenol, but 5 ml for phenol. To extract a moderately polar analyte ($\log P_{ow}$ in the range 1–3) from a sufficient volume, it is possible to increase the amount of sorbent. But even with 1000 mg of sorbent, the sample volume is around 40 ml for phenol. The limitation of utilizing C_{18} silicas for the extraction of polar organics is clearly shown.

From an analytical point of view, it is important to know accurately the $\log P_{ow}$ for pesticides and degradation products. Although these data are needed in the commercialization of a new pesticide, in many countries they remain confidential. As seen in Chapter 1 and in the review by Noble [102], large differences in values for this partition coefficient exist in the literature, depending on whether the $\log P_{ow}$ were measured by the shake-flask method, estimated from some chromatographic measurements, or calculated by some other means. Moreover, the relationship between $\log k'_w$ and $\log P_{ow}$ has been shown for $\log k'$ values extrapolated from linear curves of $\log k'$ versus methanol content, and seems to be less valid for polar compounds with $\log P_{ow}$ values lower than 2. It was shown above that quadratic extrapolation gave values closer to the real values.

For above reasons, the $\log k'_w$ values were measured or estimated, using well defined LC conditions, for the more polar pesticides and the transformation products in each class of pesticides [85]. The results are given in Table 4.7. In general, the $\log k'_w$ values were found to be higher than the $\log P_{ow}$ values.

TABLE 4.7
CLASSIFICATION OF THE MORE POLAR PESTICIDES AND/OR TRANSFORMATION
PRODUCTS BY INCREASING POLARITY WITHIN THE MAIN GROUPS OF PESTICIDES

Solute	$\log P_{ow}$	$\log k'_w$
<i>Carbamate pesticides</i>		
Aldicarb sulfoxide	-1	—
Aldicarb sulfone	-0.6	—
Oxamyl	-0.47	1.9 ± 0.1
Methomyl	0.1	1.7 ± 0.1
3-OH-Carbofuran		1.9 ± 0.1
3-OH-7-Phenolcarbofuran		1.9 ± 0.1
3-Keto-carbofuran		2.0 ± 0.1
3-Ketocarbofuran-phenol		2.0 ± 0.1
Aldicarb	1.1–1.6	2.3 ± 0.1
Carbendazim	1.4–1.6	2.2 ± 0.1
Carbetamide	1.6	2.4 ± 0.1
Carbofuran	1.2–2.3	2.9 ± 0.2
Aminocarb	1.7	3.0 ± 0.2
Propham		3.2 ± 0.2
Carbaryl	2.3–2.8	3.1 ± 0.2
Captan		>3.5
Methiocarb	2.9	>4
Chloropropham	3.1	>4
Desmedipham		>4
<i>Phenylurea pesticides</i>		
Fenuron	0.9–1.0	2.1 ± 0.1
Metoxuron	1.6	2.6 ± 0.1
Monuron	1.5–2.1	2.7 ± 0.1
Monolinuron	2.3	>3
Chlortoluron	2.3	>3
Isoproturon	2.25	>3
Diuron	2.8	>3
Neburon	—	>3
<i>Triazine and triazinone pesticides</i>		
Hydroxy-de-isopropyltriazine		1.0 ± 0.1
De-isopropylde-ethyltriazine		1.3 ± 0.1
Hydroxy-de-ethyltriazine	0.4	1.5 ± 0.1
Deisopropyltriazine	1.5	2.1 ± 0.1
De-ethyltriazine	1.5	2.6 ± 0.1
Hydroxytriazine		2.5 ± 0.1
Simazine	1.5–2.3	3.0 ± 0.2
Cyanazine	1.6–2.3	2.9 ± 0.2
Atrazine	2.2–2.8	3.4 ± 0.2
Desmetryn	2.5	>3.5
Simetryn	2.6–2.8	>3.5

TABLE 4.7 (CONTINUED)

Solute	$\log P_{ow}$	$\log k'_w$
Hydroxypropazine		>3.5
Hydroxyterbutylazine		>3.5
Prometon	3.0–3.1	>3.5
Propazine	2.9–3.0	>3.5
Sebutylazine		>3.5
Terbutylazine	3.0–3.1	>3.5
Metamitron	0.8	2.1 ± 0.1
Metribuzin	1.7	2.9 ± 0.2
Hexazinone		>3
<i>Other polar pesticides</i>		
Chlorsulfuron	–0.4–1.0	2.5 ± 0.2
Chloridazon	1.1–2.2	2.0 ± 0.1
Bentazone	–0.45	2.8 ± 0.2
Bentazone-8-OH		2.6 ± 0.2

The range of water–octanol partition coefficients is given from Ref. [102]. Extrapolated retention factors in water, $\log k'_w$, have been estimated from the relationship between $\log k'$ and the percentage of methanol in the mobile-phase measurements using analytical columns packed with C_{18} silicas.

The more polar pesticides are found among the carbamate group. A post-column reaction and a sensitive fluorescence detection can be performed for the methyl carbamates, so that the sample volume required for low-level detection is no more than 100 ml for off-line determination. Other polar pesticides which are characterized by $\log k'_w$ values lower than 2.5 are found in each main group and include numerous degradation products. The C_{18} silica will not be able to extract these analytes from a sufficient volume for trace analysis and, therefore, other sorbents will have to be selected.

Emphasis was given above to the determination of polar pesticides and/or degradation products, but some analyses are more devoted to hydrophobic compounds. Organochlorine insecticides are often analyzed at a trace-level in surface water and also in other aqueous samples such as some medicinal plant infusions. Breakthrough volumes are much higher than 1 l for these compounds which are usually analyzed by GC with electron capture detection. In that case, C_8 or lightly loaded C_{18} cartridges can be used. The recoveries were found to be similar, using cartridges packed with 500 mg of C_8 or C_{18} silica and handling 200 ml of aqueous sample [103]. There may be an advantage in using C_8 instead of C_{18} , since fewer (polar) interferences will be co-extracted.

Desorption conditions. The lower the desorption volume is, the higher the enrichment factor. The elutropic series is well known for organic solvents and the elution power decreases within the series hexane, THF, ethyl acetate, methylene chloride, acetone, acetonitrile and methanol. However, most of the medium-polarity analytes are not or are just slightly retained with pure methanol or acetonitrile, which

are often preferred because they are water-miscible. The flow rate during this step has to be low and the minimum volume is around twice the void volume of the cartridge, i.e., 0.3 ml/100 mg of C_{18} sorbents. Current volumes are between 2 and 5 ml/500 mg of C_{18} sorbent. Ethyl acetate was found to be efficient, and many apolar to moderately polar pesticides were eluted in the first 60 μ l of eluate from cartridges containing 100 mg of C_{18} silicas with recoveries higher than 90% [104,105]. The solubility of compounds in the mobile phase plays an important role in reversed-phase chromatography [95] and it is a useful guide for selecting the eluting organic solvent. Many hydrophobic compounds have a limited solubility in methanol or acetonitrile and a large volume, around 5 ml, is sometimes required for analyte elution. As a general rule, the desorption is more efficient if it is done in two or three steps of 1 ml instead of one step with 2 or 3 ml, owing to the void volumes of cartridges or disks. Desorption from disks is always achieved in two steps with 5–10 ml portions of the eluting solution, with the vacuum being interrupted so as to allow it to soak the disk for several minutes.

When the subsequent analysis is performed by GC, one method consists of eluting the analytes from the C_{18} cartridge with a GC-compatible solvent, after drying it. Another option is the desorption with a water-miscible solvent, evaporation to dryness, and re-dissolution in a GC-compatible solvent. In the first option, some differences in recoveries were observed when pure hexane, and hexane with 15% of methylene chloride, were used for the desorption after percolation of 200 ml of an aqueous sample spiked with organochlorine pesticides [103]. For example, recoveries of δ -HCH, β -endosulfan and endrin aldehyde increased from 43, 31 and 6% to 105, 96 and 83%, respectively. The addition of methylene chloride increases the solubility of the analytes and helps to give better contact with the sorbent because traces of waters are still present. Desorption with hexane alone may lead to bad recoveries and should be avoided. Acetone and ethyl acetate are more appropriate solvents for desorption and further GC analysis since the latter forms an azeotrope with water which can be removed during the evaporation to dryness. Anhydrous sodium sulfate can also be added to remove traces of water prior to the introduction of samples into the GC apparatus.

In the SPE sequence, air-drying is often applied before analyte desorption in order to remove residual water which lowers the volatility of the eluted solvent. This is important when the eluate has to be evaporated because it is incompatible with the solvent required for further clean-up or for injection into the chromatograph. This step was optimized as part of the determination of trace quantities of selected pesticides, resulting in a complete automation of cartridge conditioning, sample loading, cartridge air-drying, analyte elution, eluate blow-down, sample dilution, and sample injection steps, with a total analytical time of 30 min and with detection in the range 0.02–0.9 μ g/l [106]. The use of a workstation for the automated SPE of herbicides for GC-MS has been used successfully in recent years for the survey of many herbicides in midwestern USA [107].

Fractionation in a polarity group during the desorption is difficult and is not often reported. It should consist of step-elution with a methanol–water sequence and then a methanol–methylene chloride sequence. Such a fractionation is described as a toxicity directed method for fractionating non-polar organic toxicants in phase II of the EPA's "Methods for aquatic toxicity identification evaluations" [108]. A sequential desorption has been described for the determination of alachlor and its major metabolite, ethanesulfonic acid, in water with detection by an immunoassay [109]. Alachlor and its metabolite were isolated from water with a C_{18} sorbent and eluted sequentially with ethyl acetate and methanol. This combination is not a sequential elution by increasing elutropic power, but is explained by the fact that alachlor is very soluble in ethyl acetate while the anionic metabolite is not. Thus the latter remained adsorbed on the C_{18} sorbent and was eluted later with methanol.

Reversed-phase sorbents are often used for preconcentration of ionizable compounds in their molecular forms. Desorption from these sorbents can be performed by a solution adjusted to a pH where the analytes are in their ionic form (two units below or above the pK_a).

Blanks of C_{18} cartridges and disks. In the past, it was often pointed out that cartridges were not reproducible from batch to batch and that some impurities were released from both the sorbent and the plastic of the cartridge [110]. Now the situation has changed and there has been a real effort from the manufacturers to provide cleaner sorbents and cartridges as well as reproducible sorbents. However, some interferences from impurities can be generated by the sorbent materials, the disks of cartridge containers, and the eluting solvents used. In addition, in a blank run with "purified" water (named LC-grade water), it is clear that some interferences come from the water itself. It is easy to control the purities of the solvents by simply evaporating the desorption solvents to dryness and by GC analysis of the extracts. The impurities coming from the blank water can be also checked by changing the reference water. Some interferences have been identified in the extract by GC-MS after desorption with different solvents such as benzene, methanol, ethyl acetate or acetonitrile [72,110–112]. In addition to the common phthalates, various plasticizers have been identified which have been added purposely or are present in the polymer components. This is a result of the use of polymeric housings in the manufacture of the bonded-phase materials, either in cartridges or disks. Various alkanes (C_{18} – C_{29}) were also found as interferences both in the disks and in the cartridges [110,112]. In the cartridges, the antioxidant butylated hydroxytoluene (BHT) was reported [110, 112] whereas in the disks another antioxidant Nonox A has been found [111]. Dimethyloctadecylsilanol was identified, coming both from the C_{18} phase itself and from the C_{18} cartridge. A higher amount was observed after activation and washing by ethyl acetate than after activation and elution with methanol [112]. However, the amount of these impurities coming from the sorbents and containers is small and their effects mainly occur with sensitive GC-MS detection. After percolation of 200–500 ml using double disks of C_{18} material, no interferences were noticed using deri-

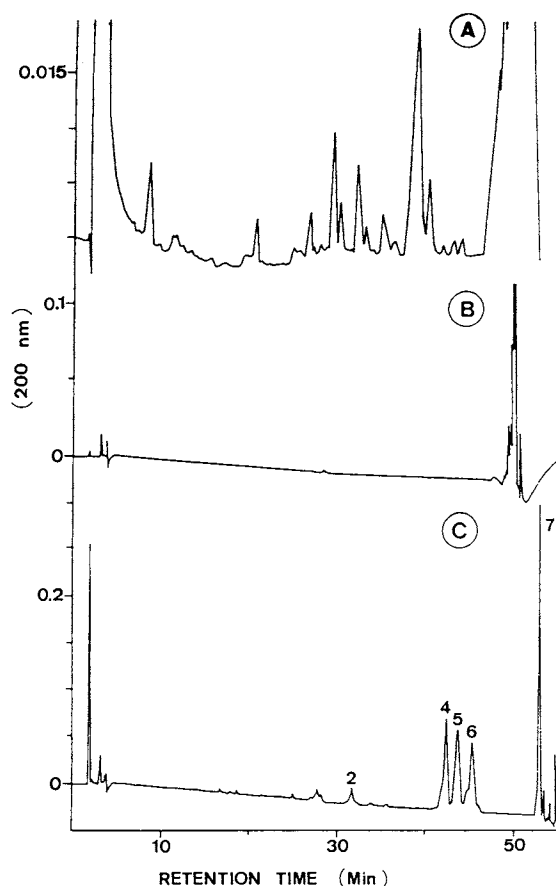


Fig. 4.12. (A) blank of C_{18} EmporeTM extraction disk obtained after preconcentrating 5 l of LC-grade water; LC-DAD at 200 nm, water, pH 1; (B) blank of SDB EmporeTM disk obtained after preconcentration of 5 l of LC-grade water at pH 0.7; (C) LC-DAD at 200 nm after preconcentration on SDB EmporeTM disk of 5 l of Ebro river water (pH 0.7) spiked at 0.25 $\mu\text{g/l}$ of (2) benazolin, (4) bentazone, (5) 2,4-D, (6) MCPA, (7) MCPP. From Ref. [72]. Analytical column, Lichrocart cartridge 60 RP-8 select B (25 cm \times 4.6 mm i.d.); acetonitrile gradient with an aqueous formic acid solution at pH 2.9.

vatization and GC-ECD, and the limits of detection for this technique vary from 0.04 to 0.4 $\mu\text{g/l}$ [72]. This is not particularly surprising, since this technique combines derivatization with ECD detection.

One must be aware that the C_{18} bonded silica is not stable at pH 1 when preconcentrating 5 l of water, partly due to the preconcentration time of 150 min needed. Decomposition of the sorbent occurs, and blank interferences were noticed on desorption (see Fig. 4.12A). In contrast, when using SDB disks – even at pH 0.7 – the blanks were quite clear, as can be seen from Fig. 4.12B. These chromatograms

clearly show the high chemical stability of the SDB sorbent over the pH range 0.7–13, with no decomposition of the sorbent material. The chromatogram in Fig. 4.12C shows that the amount of interferences from C₁₈ silica would not allow detection of the acidic pesticides at the low $\mu\text{g/l}$ level. However, lower volumes are usually handled.

Applications to multiresidue analysis of pesticides. Many applications can be found in the literature and in the brochures dealing with environmental analysis that are regularly published by the companies which commercialize SPE cartridges. Both group analysis and multiresidue approaches are carried out, and evidence for matrix interference from the sample nature can be observed in the numerous examples.

Recoveries and breakthrough volumes are often measured or estimated using LC-quality water and can be modified when salts are added (e.g., in sea water) or when many other organic compounds are present (e.g., highly contaminated surface water). SPE and LLE have been compared for the determination of neutral pollutants chosen over a wide range of solubility and polarity characteristics. The cartridge extractions of spiked waste water samples yielded recoveries on the same samples using EPA Method 625 and liquid–liquid extraction. The recoveries for triazine herbicides and organophosphorus pesticides were found to be equivalent with 1 l of spiked sea, tap and lake water samples, extracted by SPE with C₁₈ sorbents [113].

The potential for determining many pesticides over a wide range of polarity in drinking water at the low 0.1 $\mu\text{g/l}$ level is shown below [114]. The group of triazines and phenylureas was selected because they include some polar analytes such as the degradation products of atrazine, i.e., de-isopropylatrazine, hydroxyatrazine and de-ethylatrazine, and fenuron or metoxuron (with $\log k'_w$ below or around 2.5, see Table 4.7), many moderately polar ones and rather apolar pesticides such as neburon ($\log P_{ow} = 4.3$, according to Ref. [84]). The analytical separation was carried out by reversed-phase chromatography using a C₁₈ analytical column and an acetonitrile gradient in phosphate buffer at pH 7. The separation was not optimized because the occurrence of each compound in the same sample is unlikely. Only some target compounds have to be well separated on the basis of their amount of usage. In addition, as can be seen in Fig. 4.13a, co-eluted analytes do not belong to the same group and can easily be differentiated by the UV diode array detector (DAD). The chromatogram, Fig. 4.13a, corresponds to the direct injection of the mixture containing 5 ng of each pesticide, showing that the limits of detection (LOD), defined as a signal–noise ratio of 3 are between 0.05 and 3 ng. From the LOD values obtained by direct injection, one can easily calculate the volume that has to be preconcentrated on the basis of a 100% recovery and a required limit of detection in concentration units in the sample of 0.01–0.02 $\mu\text{g/l}$. This LOD is required for reliable quantification at the 0.1 $\mu\text{g/l}$ level in drinking water samples. As an example, with a LOD of 2 ng obtained by injection of 100 μl of the extract (the maximum volume that can be injected into a 25 cm-long analytical column without loss of efficiency), and taking into account the fact that the extract cannot usually be dissolved in a volume lower

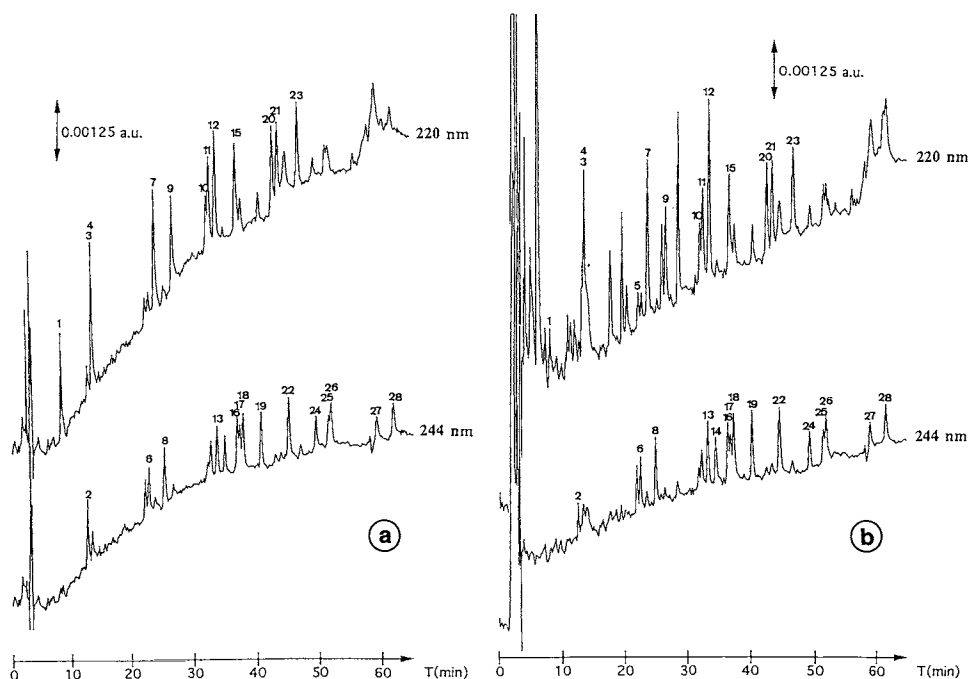


Fig. 4.13. (a) Direct injection of a mixture of triazine and phenylurea pesticides (5 ng of each analyte via a 50 μ l loop); (b) Injection of 50 μ l of an extract of drinking water spiked with 0.1 μ g/l of each analyte. Preconcentration of 500 ml of drinking water via a 500 mg C_{18} silica cartridge, desorption with 4 ml of methanol, evaporation to dryness, and addition of 500 μ l of an acetonitrile–water mixture (20:80, v/v). Analytical column, Supelcosil LC-18-DB 25 cm \times 4.6 mm i.d.; acetonitrile gradient with 0.005 M phosphate buffer at pH 7; UV detection at 220 nm. Peaks: (1) DIA; (2) fenuron; (3) OHA; (4) DEA; (5) hexazinone; (6) metoxuron; (7) simazine; (8) monuron; (9) cyanazine; (10) metabenzthiazuron; (11) simetryn; (12) atrazine; (13) chlortoluron; (14) fluometuron; (15) prometon; (16) monolinuron; (17) isoproturon; (18) diuron; (19) difenoxuron; (20) sebutylazine; (21) propazine; (22) buturon; (23) terbutylazine; (24) linuron; (25) chlorbromuron; (26) chloroxuron; (27) diflubenzuron; (28) neburon. From Ref. [114] with permission.

than 200–500 μ l, depending on the sample matrix, a simple calculation indicates that the sample volume to be handled should be in the range 300–500 ml. Figure 4.13b shows the chromatograms at 220 and 244 nm obtained for an extract from 500 ml of drinking water which was spiked with 0.1 μ g/l of each pesticide, after dissolving the dry extract in 500 μ l of mobile phase and injecting a 50 μ l aliquot into the analytical column. If recoveries are 100%, the amount in the injected extract is the same as that injected in Fig. 4.13a. Apart from the early-eluted peaks 1–4, for which the recoveries are respectively 26, 51, 68 and 68%, one can see that the peak heights which cor-

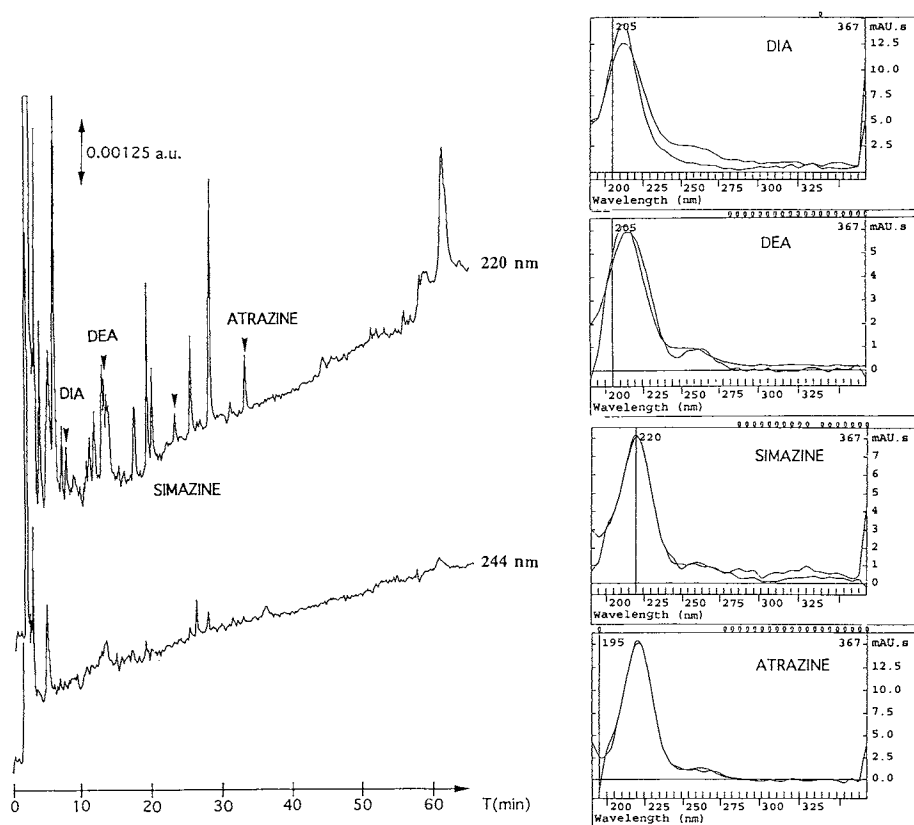


Fig. 4.14. Injection of 50 μ l of an extract of drinking water (similar sample to that in Fig. 4.13b). The same procedures were used for extraction and analysis as described in Fig. 4.13b. Identification by the DAD detector. From Ref. [114] with permission.

respond to the spiked pesticides are similar in Fig. 4.13a,b. Peaks 7 and 12 are even higher, reflecting the presence of these compounds in the sample, as shown in Fig. 4.14 where a non-spiked sample was analyzed under the same experimental conditions. The occurrence of simazine (peak 7) and atrazine (peak 12) are confirmed by comparison of retention times and UV spectra from the library of the DAD at respective concentrations of $0.016 \pm 0.003 \mu\text{g/l}$ and $0.12 \pm 0.02 \mu\text{g/l}$. The match between the retention times and the two UV spectra is excellent, so no further confirmation is required. The peaks which show up at 7.9 and 13.3 min can be de-isopropylatrazine and de-ethylatrazine, but the match is not excellent and another method should be required for confirmation.

Various examples of multiresidue analysis using C_{18} membrane disks have also been reported (see the references cited in Ref. [72]). Table 4.8 shows the average percent recovery values obtained at spiking levels of 1–5 $\mu\text{g/l}$ of river water, using

Empore™ extraction disks containing C₁₈ material, and comparison with dichloromethane LLE according to the National Pesticide Survey (NPS) method 4 [33]. For complete extraction of the most acidic herbicides, e.g., bentazone, sulfuric acid (pH 2) and 50 ml of dichloromethane were added to the water sample, which was shaken again and extracted. From Table 4.8 we can conclude that the recoveries from a 1 l sample with the Empore™ extraction disk are similar to those with the LLE procedure, thus indicating that the SPE method offers data of as good quality as that NPS Method 4, since the NPS and US EPA accept a method yielding recovery values between 70 and 130% for samples analyzed at the $\mu\text{g/l}$ level. Few values fail to accomplish this criterion, e.g., the most polar pesticides (aldicarb sulfoxide and sulfone, 3-keto carbofuran phenol) for which breakthrough has occurred with a volume of 1 l, and aldrin because of adsorption problems. Using a C₁₈ 90 mm Empore extraction disk, recoveries were measured for 18 pesticides with 10 l of water samples. The more polar components of the mixture were metribuzin and simazine, with respective recoveries of 32 and 70% with spiked LC-grade water [76].

The previous examples have clearly shown that the limits of detection are in the range 0.01–0.05 $\mu\text{g/l}$ for analytes over a wide range of polarity with a 500 ml sample extracted using a 500 mg C₁₈ silica cartridge. The limitation of C₁₈ silica only exists for the extraction of “polar” pesticides or degradation products, since loss in recoveries occurs from the breakthrough of these analytes.

Application to non polar pesticides. No breakthrough should occur for apolar pesticides ($\log P_{\text{ow}} > 4$) with the handling of at least 1 l. However, recoveries reported in the literature are sometimes low. Another cause of loss in recoveries has been observed, which is not due to breakthrough, but to adsorption of these hydrophobic compounds onto connecting tubes and containers. The adsorption of some non polar pesticides onto glass and Teflon bottles has been compared [115]. Samples were spiked at the 0.25 $\mu\text{g/l}$ level and recoveries ranged from less than 20% for permethryn, cypermethryn, fenvalerate and DDE, between 30 and 60% for DDD and DDT, above 80% for HCH, dieldrin and endrin, and 100% for atrazine and simazine. Adsorption was in general higher on Teflon than on glass bottles.

Such problems have been encountered for pesticides which combine high hydrophobicity with very low water solubility, such as most of the organochlorinated pesticides, most of the pyrethroids, some organophosphorus pesticides, and some diniroaniline herbicides (trifluralin and pendimethalin). In order to avoid the adsorption problems, one solution is to add a small proportion of organic solvent (methanol, acetonitrile or isopropanol) to the samples before percolation through the cartridge. Since for apolar compounds the breakthrough volumes are very high, the reduction of breakthrough volume from adding 5–10% of an organic solvent can still allow the handling of 500 ml of sample without breakthrough and consequent loss in recoveries. An automated apparatus was used for the extraction of pyrethroid pesticides using C₁₈ cartridges packed with 100 mg of sorbent [116]. With a sample volume of 27 ml spiked with 2 $\mu\text{g/l}$, the effect of the added methanol on recoveries was studied.

TABLE 4.8
AVERAGE PERCENT RECOVERY AND COEFFICIENT OF VARIATION (CV) IN RIVER WATER
USING C₁₈ EMPORE EXTRACTION DISKS AND DICHLOROMETHANE LLE (FROM REF. [72])

Pesticide	Empore disk		LLE	
	R (%)	CV	R (%)	CV
Aldicarb	83	7	62	10
Aldicarb sulfoxide	35	21	18	24
Aldicarb sulfone	30	20	36	18
Aldrin	51	10	n.d.	
Atrazine	100	5	99	4
Bentazone	72	6	81	7
Captan	85	7	n.d.	
Captafol	110	3	n.d.	
Carbaryl	87	8	109	2
Carbendazim	40	25	n.d.	
Carbofuran	74	7	90	4
γ-Chlordane	72	3	n.d.	
Chlorothalonil	107	6	n.d.	
Chlorpyrifos	94	4	86	3
Cyanazine	84	7	92	14
4,4'-DDE	66	5	n.d.	
4,4'-DDT	79	2	n.d.	
De-ethylatrazine	76	13	90	9
Dieldrin	93	6	n.d.	
Endrin	86	4	n.d.	
Ethirimol	120	25	n.d.	
Fenamiphos	95	5	87	9
Fenamiphos sulfone	97	11	98	10
Fenamiphos sulfoxide	100	5	97	6
Folpet	68	6	n.d.	
3-Ketocarbofuran phenol	60	19	89	6
Linuron	96	6	94	5
Metalaxyl	104	3	n.d.	
Propanil	95	7	94	3
Simazine	80	10	97	8
Tetrachlorvinphos	96	4	92	4
Vinclozolin	108	4	n.d.	

Spiking level, 1–5 µg/l (*n* = 5) for each pesticide Water volume, 1 l.

It was shown that the addition of 30% methanol was required in order to remove any process of adsorption and to obtain recoveries around 90% for the pyrethroids fenpropathrin, permethrin and deltamethrin. Another example is for the determination of organochlorinated pesticides with the handling of 200 ml on C₈ silica cartridges, where up to 25% of acetone was added without any loss in recoveries [103]. Another example deals with the extraction of organo-chlorine, -nitrogen and -phosphorus

pesticides for a multiresidue analysis using GC with simultaneous NPD and ECD detection. After glass-fibre filtration, 10 ml of methanol was added to 1 l of water samples before percolation through a 500 mg cartridge [117]. Desorption was achieved with 3 ml of acetone after connection to a column packed with anhydrous sodium sulfate to remove traces of water. Under these experimental conditions, recoveries were measured for 82 pesticides: the lowest ones were for some polar nitrogen pesticides (de-ethylatrazine and de-ethylterbutylazine, at 7 and 40%) and for the more apolar organochlorinated pesticides. Recoveries below 70% were obtained for hexachlorobenzene, *o,p*-DDE, *p,p'*-DDE, *o,p*-DDT, and *p,p'*-DDT. The amount of added methanol is only 1% which is sufficient for avoiding the adsorption of many pesticides.

Some of these non-polar pesticides are also those which are easily adsorbed on particulate matter and humic acids. One must still be aware of the loss due to pre-filtration. An interesting example of the problem that may occur with the use of disks is seen in the preconcentration of aldrin (reported $\log P_{ow}$ in the range 5.9–7.4) [63,67–70]. Recoveries in various natural, ground and drinking waters, spiked at levels of 0.1–1 $\mu\text{g/l}$, have varied from 37 to 65%. The lower values correspond to natural (37%) and ground waters (45%) having a higher amount of particulate matter. Experiments indicated that the low recoveries could not be attributed to losses from adsorption on the glassware, but came in the prefiltration processes. One solution is to achieve pre-filtration and extraction on disks in the same run, by adding layers of filters above the C_{18} disks and to desorb both the filtering medium and the C_{18} disks [40]. Special filter aids to put on top of the disks are also available.

A recent study reported the recoveries of herbicides as a function of the dissolved organic carbon (DOC) content from humic acids using solid-phase extraction disks [118]. The pesticides studied were some triazines (atrazine, simazine, cyanazine, metribuzin), two organophosphate pesticides (methylparathion and profenofos), acetanilides (alachlor and metolachlor), dinitroanilines (trifluralin and pendimethalin), propanil and norflurazon. Except for the dinitroanilines, no significant reductions in recovery were obtained up to an amount of DOC of 10 mg/l, and a very slight one was obtained with a content of DOC of 25 mg/l, all the recoveries still being in the range 78–89% at pH 6 and 8. Although the authors concluded from the different recoveries obtained at pH 6 and 8 that these compounds were slightly bound to humic acids by non-polar interactions, the results first indicated that all these compounds are mainly in the water. The recoveries of pendimethalin and trifluralin were 63 and 50%, respectively, when no humic acids were added and then decreased to around 50 and 45% when the amount of DOC increased to 25 mg/l. The results show that these two pesticides -which are effectively the most hydrophobic ones and have much lower water solubility than the others- were adsorbed onto humic material by hydrophobic interactions. However, the low recoveries obtained when no humic material was added clearly indicated a loss, certainly because the

compounds adsorb to the stainless steel reservoirs and tubing. Again, one has to take great care in the analysis of hydrophobic pesticides.

This addition of organic solvent cannot be done in the case of multiresidue analysis including both very polar and non-polar analytes because it will cause breakthrough of the polar species. When the sample contains both very polar pesticides and degradation products and very apolar ones, it is impossible to have good recoveries over the whole range; one solution is to split them into polar and moderately polar compounds on the one hand and into apolar ones on the other hand. Another practical problem is linked to the determination of both polar and apolar pesticides. In the subsequent analysis by LC, the separation of the more polar analytes will require an initial LC mobile phase containing a large proportion of water. Using the initial mobile phase will not ensure complete dissolution of the more apolar compounds in the mixture. Using a high proportion of organic solvent, or pure organic solvent, may allow the complete dissolution of all the compounds, but the injection of 50–100 μ l of an aliquot will induce bad peaks in the chromatogram. This may be another reason for considering the trace analysis of polar to moderately polar pesticides in one run and the more apolar in another run.

Matrix effects and pH of the samples. There is a real interest in the simultaneous determination of acidic and neutral herbicides in ground and surface waters, since they are often applied together. This is shown by the results of some surveys [119–123]. A 5-year monitoring of rivers from Brittany (France), indicated that atrazine, simazine, diuron and isoproturon were detected very often, but that the acidic herbicides such as bentazone, bromoxynil, mecoprop and dinoterb were also frequently detected [122]. In the literature, because of the old scheme that was used when LLE was the only extraction method, there is often, even now, a fractionation into the analysis of acidic pesticides on the one hand, and the sum of neutral and basic ones on the other [124–127]. Most of the reported multiresidue analyses including acidic and neutral pesticides have been performed using on-line techniques [33, 128–131].

Acidic herbicides are not, or are only slightly retained by C_{18} silica in their ionic forms so they can be extracted using a C_{18} silica cartridge provided the sample has been acidified before the percolation. Table 4.9 shows the low recoveries of extraction measured for some acidic herbicides when 500 ml of drinking water was percolated at pH 7 or acidified to pH 2 or 3 with perchloric acid, and spiked at 0.5 μ g/l. At pH 7 the recoveries are low, and for very acidic herbicides it is necessary to acidify the samples to pH 2. One is not recommended to use C_{18} silica cartridges at pH below 2 because of the solubility of the silica. When natural water samples are acidified to pH 2 there is an interfering peak caused by humic and fulvic acids, as shown in the chromatograms in Fig. 4.15. In recent studies, it has been shown that the strongly acidic characteristics (pK_a 3.0 or less) was due half to keto acids and aromatic carboxylic group structures, and half to aliphatic carboxyl groups in unusual and/or complex configurations [132,133]. This strong acidity explains why the inter-

ferences are only detected at acidic pH and are more important at pH 2 than at pH 3. If one takes into account this co-extraction of humic and fulvic acids and optimizes the mobile-phase gradient in order to elute the first compounds after the interfering peak, most of the pesticides can still be determined at the $0.1 \mu\text{g/l}$ level in drinking water samples. As shown in Fig. 4.15a, only the very polar compounds show up in the interfering peak if the mobile-phase gradient is adjusted so that most of the peaks are eluted after 20 min. Note that the shape of this interfering peak depends on the shape of the gradient applied and it can also appear in the middle of the chromatogram as a large hump [33,128–131,134]. With the surface water sample (Fig. 4.15b), the spiked level is five times higher, i.e., $0.5 \mu\text{g/l}$, and the attenuation of the DAD is also higher. One can see the consequence of the humic and fulvic interferences because the detection limits, which are in the $0.1 \mu\text{g/l}$ range in drinking water, are closer to $0.5 \mu\text{g/l}$ in a contaminated surface water. It will be seen later that detection limits can be improved with an additional clean-up step.

The analysis of more than 30 phenoxycarboxylic acids and other acidic pesticides was presented in tap, ground, surface, and sewage water at the low ng/l level. The extraction was achieved with 1 l of sample acidified at $\text{pH} < 2$ with HCl using a 1 g C_{18} cartridge. Then the detection was performed after pentafluorobenzoylation using GC-MS. No effect of the sample matrix was observed owing to the selective derivatization step and detection. The main difference from the method described above is the limitation to a group of pesticides that can be thus analyzed [126].

4.2.3.3.2. Apolar copolymer sorbents. During the last 20 years, Amberlite XAD-type copolymers (mainly XAD-2) have certainly been the sorbents most used for off-line trace enrichment of environmental samples. These styrene-divinylbenzene (SDB) resins exist with a range of surface areas, granulometry, and pore size and their ability for preconcentrating non-polar to relatively polar analytes has been widely demonstrated [135–147]. The greatest disadvantage of these resins is the gen-

TABLE 4.9
RECOVERIES (%) OF ACIDIC PESTICIDES AT DIFFERENT pH (FROM REF. [114] WITH PERMISSION)

Compound	$\text{p}K_{\text{a}}$	pH 2	pH 3	pH 7
Dicamba	1.94	89	46	2
Bentazone	3.2	100	100	6
Ioxynil	3.96	98	83	31
MCPP	3.07	104	108	27
2,4 DB	4.8	98	92	38
2,4,5 TP		100	78	10
Dinoterb	5.0	72	49	30

Preconcentration of 500 ml of drinking water spiked with $0.5 \mu\text{g/l}$ of each analyte on a 500 mg C_{18} cartridge.

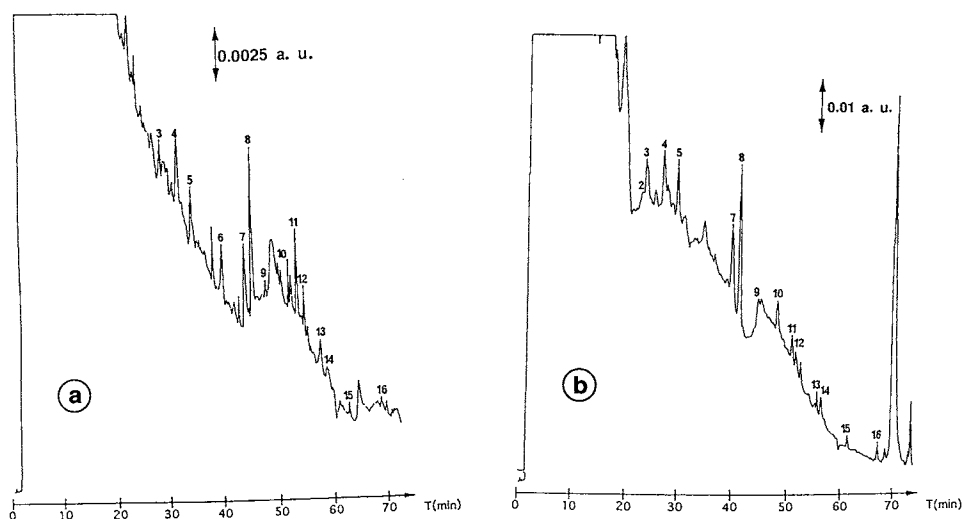


Fig. 4.15. Effect of the matrix sample when the samples are at pH 2; (a) injection of an extract from drinking water spiked with $0.1 \mu\text{g/l}$ of each analyte and (b) of an extract from the River Seine spiked with $0.5 \mu\text{g/l}$ of each analyte. From Ref. [114] with permission. Preconcentration of 500 ml via a 500 mg C_{18} silica cartridge, desorption with 3 ml of methanol, evaporation to dryness, and addition of 500 μl of an acetonitrile–water mixture (20:80, v/v). Analytical column, Bakerbond Narrow Pore C_{18} silica, 25 cm \times 4.6 mm i.d.; acetonitrile gradient with 0.005 M phosphate buffer at pH 3, UV detection at 220 nm. Peaks: (1) chloridazon; (2) aldicarb; (3) metoxuron; (4) simazine; (5) cyanazine; (6) benta-zone; (7) atrazine; (8) carbaryl; (9) isoproturon; (10) difenoxuron; (11) ioxynil; (12) MCPP; (13) 2,4-DB; (14) 2,4,5-TP; (15) metolachlor; (16) dinoterb.

eration of impurities that are laborious to eliminate subsequently. The resins have to be purified thoroughly before use by extraction in a Soxhlet apparatus. The sequences of flushing solvents commonly recommended are methanol, acetonitrile, ether [136]. Several purification procedures have been compared and the solvent change from methanol to water was found to be responsible for impurity release. Desorption with diethyl ether was recommended [140,148].

The fact that the XAD sorbents have to be purified before use explains why pre-packed cartridges had not been available. Until 2 years ago, the SDB sorbent had only been available in extraction disks for off-line extraction. Porous and rigid SDB polymers have been commercialized for liquid chromatography. These sorbents, PRP-1 (Hamilton) and PLRP-S (Polymer Laboratories) are pressure resistant, stable over the pH range 1–14 and exist in a small granulometry, 7 to 15–25 μm . Although they were available in bulk, they were too expensive to be packed in disposable cartridges. Several applications reported have used them in precolumns for on-line pre-concentration of pesticides and other organic contaminants (see Chapter 5). New resins have been synthesized recently by some manufacturers and have been available during these last 2 years. They have high specific surface areas, around

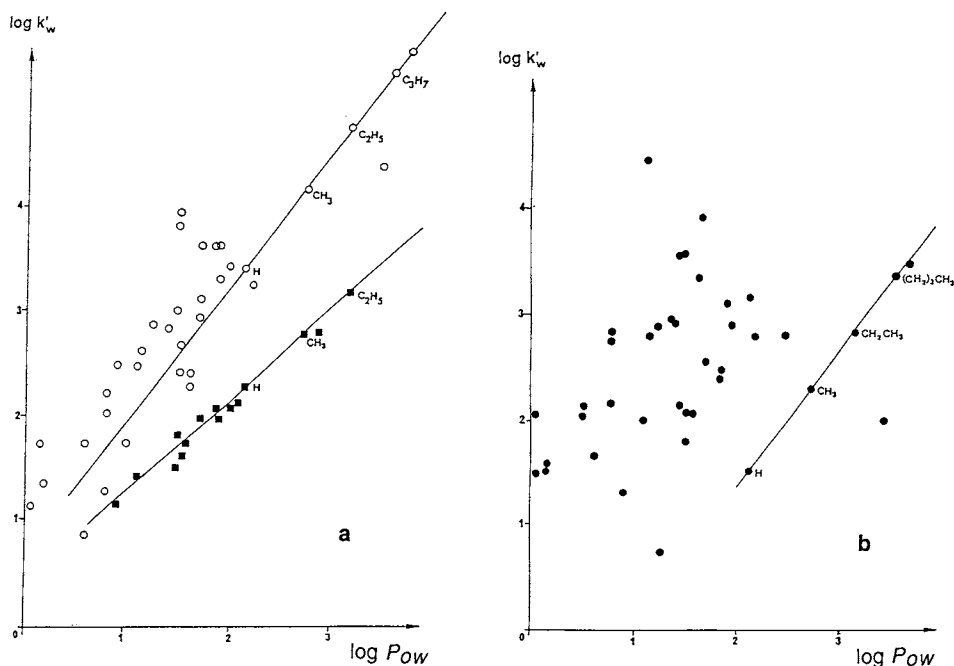


Fig. 4.16. Relationship between the octanol–water partition coefficient ($\log P_{ow}$) and the retention factors ($\log k'_w$) estimated or measured in water on (a) C_{18} silicas and PRP-1 SDB sorbent, and (b) porous graphitic carbon, Hypercarb, for various organic compounds. From Ref. [150].

1000 m^2/g , compared to LC-grade SDB stationary phases as can be seen in Table 4.10. However, these polymers certainly do not have all the required properties for use as stationary phases in LC, and are not available in analytical columns, so no chromatographic data have yet been reported.

Prediction from hydrophobicity parameters. The retention behaviour of analytes is governed by hydrophobic interactions similar to those with C_{18} silicas, but, owing to the aromatic rings in the network of the polymer matrix, one can expect strong electron-donor interactions (π – π) with aromatic rings of solutes. These should also be sensitive to the changes in the solute electron density caused by the electron-donating and withdrawing ability of solute substituents. LC data are available only for LC-grade SDB. The retention behaviour of analytes on PRP-1 sorbent has been studied and compared to the retention obtained with C_{18} silicas. First, it was shown that $\log k'_w$ could also be extrapolated from the relationship between $\log k'_w$ and the methanol content [55,149]. This is as shown in Fig. 4.16a which confirms that the primary retention mechanism is governed by hydrophobic interactions. For a set of many organic compounds, the results indicated that the solutes are about 10–40 times more strongly retained by PRP-1 than by C_{18} silicas. However, the relationship between extrapolated $\log k'_w$ values and $\log P_{ow}$ values is less linear than that existing

TABLE 4.10
CHARACTERISTICS OF SOME APOLAR POROUS COPOLYMERS USED AS LC AND
EXTRACTION SORBENTS

Sorbent	Manufacturer	S_{BET} (m^2/g)	Particle size (μm)	Type (amount)
PRP-1	Hamilton	415	5 or 10	Precolumns
PLRP-S	Polymer Lab.	550	15–60	Precolumns
Empore disk	JT Baker	350	6.8 (1.4–10.9)	Disk (47 and 90 mm i.d.)
Lichrolut EN	Merck	1200	40–120	Cartridges (200 mg)
Envi Chrom P	Supelco	900	80–160	Cartridges (250–500 mg)
SDB	JT Baker	1060	43–123	Cartridges (50–500 mg)
Isolute EN	IST	1100	–	Cartridges (200 mg)
Chromabond HR-P	Macherey-Nagel	(high)	–	Cartridges (200–1000 mg)

with C_{18} silicas [150]. The highest difference is for benzene derivatives substituted by nitro groups – having a strong electron-withdrawing effect – and the smallest for hydroxy groups – showing an electron-donating effect. The slope of the curves are not the same for C_{18} silicas and for PRP-1. The difference is higher for hydrophobic compounds than for polar ones. For $\log P_{\text{ow}}$ values below 1, the difference in retention between C_{18} silica and PRP-1 is no longer observed.

The effect of the specific surface area is important as shown in Table 4.11. In order to estimate $\log k'_w$ values in water-rich mobile phases, a small column (5 cm long \times 4.6 mm i.d.) was laboratory-packed with one of the high specific area SDB (here named HSA/SDB) polymers and another one (3 cm long \times 0.46 mm i.d.) with a stacking of small disks enmeshed with a SDB polymer (see characteristics in Table 4.10.) [151]. Data on C_{18} silica is also reported for comparison. The retention factors are similar for PRP-1 and the SDB disks, but the specific surface areas are not very different (415 and 350 m^2/g , respectively) and are higher than those observed with C_{18} silica. With HSA/SDB there is a large increase in retention, since the difference is between 1.3 and 1.8 in log units, indicating that this polymer has 20–60 times more retention power towards polar pesticides than have the polymers with lower specific areas.

The various suppliers of these HSA/SDB polymers indicate that the breakthrough volume of de-isopropylatrazine is higher than 1 l, using a 200 mg cartridge.

Desorption conditions. As a results of the primary hydrophobic retention mechanism, compounds are not – or are only slightly – retained by organic solvents, and the same eluotropic series as described for C_{18} silicas can be observed. However, compounds are more retained than on C_{18} silica, so a higher volume than twice or three times the void volume of the cartridge will be required. For a 200 mg cartridge, various examples dealing with multiresidue analysis of polar and non-polar pesticides indicate that efficient desorption is obtained with 2 \times 2 ml of a mixture of acetonitrile and methanol (50:50, v/v) [152]. Other examples are given for the desorp-

TABLE 4.11

COMPARISON OF THE LOGARITHMS OF THE RETENTION FACTORS IN WATER OBTAINED WITH C₁₈ SILICA AND VARIOUS SDB COPOLYMERS (FROM REF. [151])

Compounds	log k'_w			
	C ₁₈	PRP-1	SDB (disk)	HSA/SDB
De-isopropylatrazine	2.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.2	4.4 ± 0.3
De-ethylatrazine	2.7 ± 0.1	3.5 ± 0.3	3.5 ± 0.2	4.8 ± 0.3
Simazine	3.4 ± 0.1	>4	4.1 ± 0.2	5.9 ± 0.3
2-Chlorophenol	2.9 ± 0.1		3.6 ± 0.2	
Oxamyl	1.7 ± 0.1		2.8 ± 0.2	4.1 ± 0.3
Aldicarb	2.5 ± 0.1		4 ± 0.2	5.3 ± 0.3
Carbendazim				5.7 ± 0.3
Chloridazon	2.3 ± 0.1		3.8 ± 0.2	

log k'_w values extrapolated from the relationships between log k' and the percentage of methanol.

tion of chlorophenoxy acids which were performed from a 200 mg cartridge with 3 ml of acetone for a subsequent GC analysis after derivatization [153]. As shown below, the desorption of an hydrophobic pesticide such as alachlor was performed with 4 ml of pure methanol from a 200 mg cartridge. The use of C₁₈ silica and Envi™ ChromP were compared for the analysis of polar thermolabile pesticides and surprisingly, recoveries were found to be lower using a cartridge packed with 2 g of Envi™ ChromP than when using a 2 g C₁₈ silica [154]. However, this can easily be explained by the fact that for both columns, the desorption was achieved with 5 ml of methanol, which is a minimum volume for 2 g of C₁₈ silica (about twice the void volume) but too low for the SDB resin.

All the other considerations that were mentioned for the desorption of non-polar pesticides from C₁₈ silica cartridges are significant for desorption from these polymeric sorbents.

Application to the extraction of polar pesticides. Some applications have been presented which show the advantage of the SDB disk over C₁₈ silica for better retention of compounds such as chlorophenols and other pesticides [155,156]. The recent HSA/SDB sorbents were investigated for the extraction of very polar pesticides and degradation products [151,157]. Table 4.12 compares the recoveries obtained on C₁₈ disks by percolating 500 ml of water spiked with polar pesticides to those obtained by percolating 1 l of water spiked with the same compounds through a SDB disk or through a HSA/SDB cartridge. Recovery data for oxamyl and de-isopropylatrazine show the highest retention obtained with the new polymeric 200 mg cartridges. Only oxamyl has a breakthrough volume lower than 1 l, but the recovery is 88% and much higher than that obtained with the SDB disk (27%).

The recoveries of 13 polar pesticides (with log P_{ow} values <2) selected within the main chemical groups were measured using a 200 mg HSA/SDB cartridge and per-

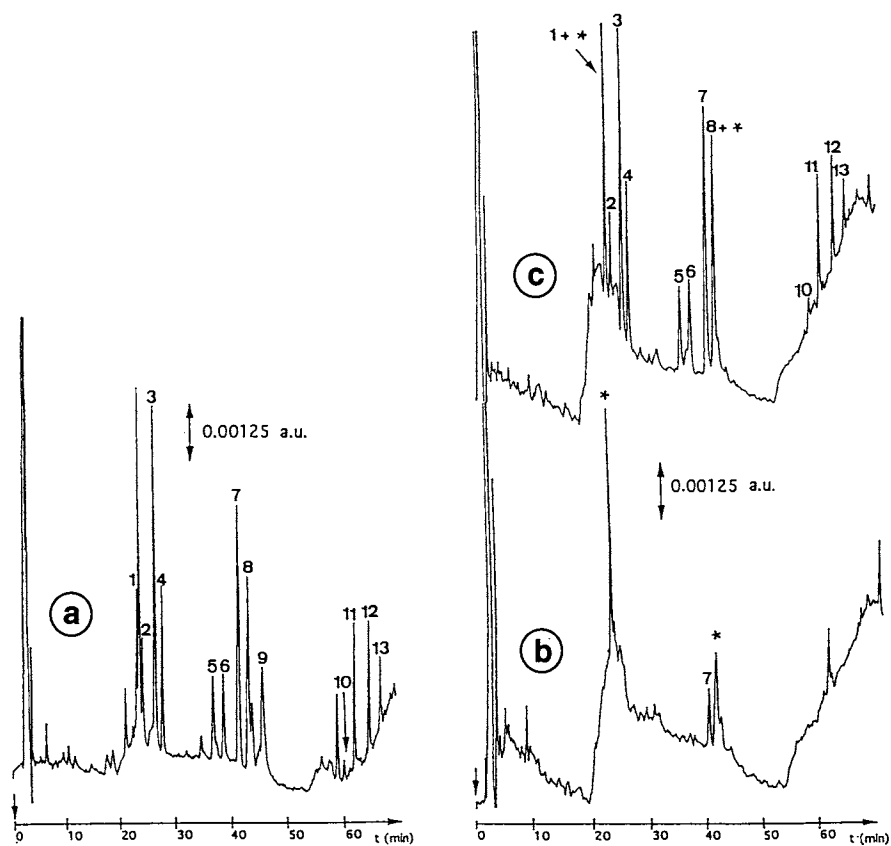


Fig. 4.17. Preconcentration of (a) 1 l of LC-grade water spiked with $0.1 \mu\text{g/l}$ of each pesticide, (b) 1 l of non-spiked drinking water sample and (c) 1 l of spiked drinking water using a 200 mg SDB-1 cartridge. C_{18} analytical column ($25 \text{ cm} \times 0.46 \text{ cm i.d.}$), mobile phase acetonitrile–water gradient from 5% of ACN to 30% at 60 min, UV detection at 220 nm. Compounds: (1) oxamyl; (2) methomyl; (3) DIA; (4) monocrotophos; (5) fenuron; (6) metamitron; (7) DEA; (8) chloridazon; (9) carbendazim; (10) aldicarb; (11) aminocarb; (12) metribuzin; (13) metoxuron. From Ref. [158].

colating 1 l of LC-grade and drinking water spiked with $0.1 \mu\text{g/l}$ of each analyte [158]. The desorption was achieved with 2 ml of a mixture of acetonitrile–water (50:50). The corresponding chromatograms are represented in Fig. 4.17. The recoveries were found to be higher than 85% for most of the compounds except oxamyl due to breakthrough on the sorbent and aldicarb (73%) and carbendazim (80%) for which it was verified that losses had occurred during the final evaporation step. Very few extra peaks were detected in the chromatogram of spiked LC-grade water and were caused by the water (this was easily verified by changing the quality of the LC-grade water), thus showing that no impurities were generated by the resin or the cartridge which can be detected by LC-UV-DAD. Apart from aldicarb, which can be

TABLE 4.12
RECOVERIES (%) OF EXTRACTION OBTAINED FOR POLAR PESTICIDES IN LC-GRADE
WATER (FROM REF. [151])

Solute	log P_{ow}	C ₁₈ disk	SDB disk	HSA/SDB cartridge
Oxamyl	-0.47	<3	27	82
De-isopropylatrazine	1.1	21	53	92
De-ethylatrazine	1.5	58	93	100
Carbendazim	1.56	62	84	88
Aldicarb	1.1-1.5	69	72	90
Simazine	1.96	95	90	94

Spiked at 0.1 $\mu\text{g/l}$ ON A 47-mm C₁₈ disk (450 mg of sorbent, J.T. Baker, sample 500 ml), 47 mm SDB disk (SDB, J.T. Baker, 450 mg of sorbent, sample 1 l) and on a 200 mg HSA/SDB cartridge (J.T. Baker, sample 1 l). Water-octanol constant log P_{ow} from Ref. [102].

detected better at 200 nm, the detection limits are in the range 0.01–0.05 $\mu\text{g/l}$ for each polar pesticide, including the very polar carbamates oxamyl and methomyl which can thus be detected without derivatization and therefore included in a multi-residue analysis.

Matrix effect: removal of the humic and fulvic interferences. Since HSA/ polymeric sorbents provide higher retention for moderately polar pesticides, the retention of acidic pesticides was studied at pH > 3 in order to reduce the amount of co-extracted humic and fulvic acids in surface waters [151]. The recoveries of the acidic pesticides which are reported in Table 4.9 using a C₁₈ silica cartridge were also measured using a 200 mg HSA/SDB cartridge and a sample volume of 500 ml of drinking water spiked with 0.1 $\mu\text{g/l}$ of the acidic analytes and adjusted to pH 7. The recoveries of dicamba which was lower than 3% on a 500 mg cartridge under the same extraction conditions was measured at 78% and the recoveries of all the other acidic compounds was found above 85–90%. Figure 4.18 shows the differences in the chromatograms when the drinking water samples are set at pH 3 (Fig. 4.18A) or at pH 7 (Fig. 4.18B). As on C₁₈ silicas, humic and fulvic interferences are co-extracted at pH 3 whereas they are not at pH 7. The fact they are still not retained at pH 7 is a result of their high polarity because of the numerous ionized groups and/or to their different configuration at pH 7 and their possible occurrence in the colloidal fraction [132,133]. However, a consequence of the high retention of acidic pesticides in their ionic form, together with the absence of retention of humic and fulvic interferences, is the remarkable possibility of determining acidic and neutral pesticides in surface water samples without any clean-up, at the low 0.1 $\mu\text{g/l}$, as shown in Fig. 4.19.

4.2.3.3.3. Carbon-based sorbents. The use of carbon-based sorbents for the extraction of compounds from aqueous media has been reviewed recently [159]. Activated carbon was certainly one of the first material used for extracting medium- to low-polarity organic compounds from water, but was superseded because of irreversible

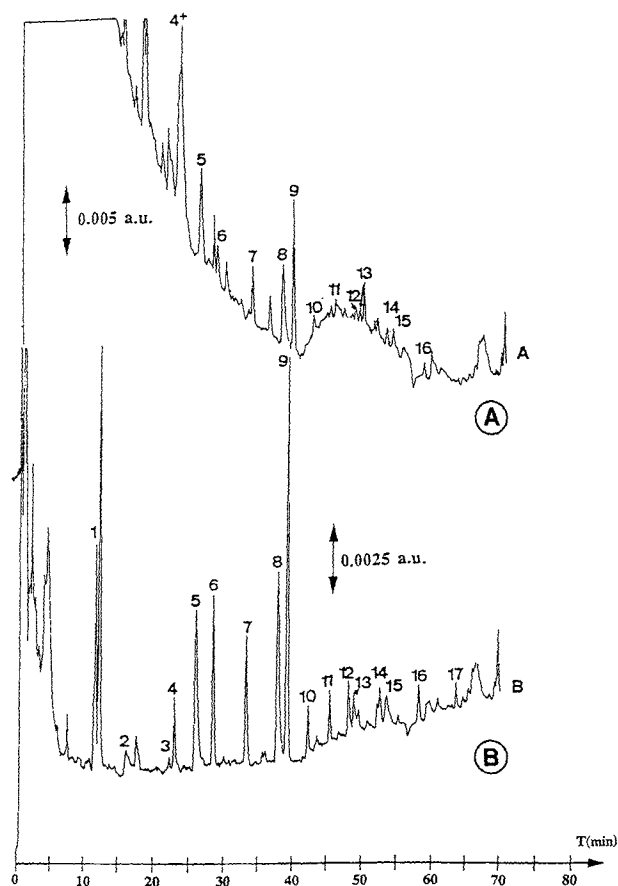


Fig. 4 18. Effect of the pH of the sample and of the matrix of the sample. (A) 500 ml of drinking water spiked with $0.1 \mu\text{g/l}$ of herbicides sample adjusted to pH 3, (B) 500 ml of drinking water spiked with $0.1 \mu\text{g/l}$ of herbicides sample at pH 7. From Ref. [151]. Preconcentration through a 200 mg SDB cartridge, desorption with 4 ml of methanol, evaporation to dryness, and addition of $200 \mu\text{l}$ of an acetonitrile–water mixture (20:80, v/v). Analytical column, Bakerbond Narrow Pore C_{18} silica, $25 \text{ cm} \times 4.6 \text{ mm i.d.}$; acetonitrile gradient with 0.005 M phosphate buffer at pH 3. UV detection at 220 nm. Peaks: (1) chloridazon; (2) dicamba; (3) aldicarb; (4) metoxuron; (5) simazine; (6) cyanazine; (7) bentazone; (8) atrazine; (9) carbaryl; (10) isoproturon; (11) ioxynil; (12) MCPP; (13) difenoxuron; (14) 2,4-DB; (15) 2,4,5 TP; (16) metolachlor; (17) dinoterb.

adsorption problems. Only a few carbon-based sorbents are available for SPE in water. The most common ones are graphitized carbon blacks (GCB) obtained by heating carbon blacks at high temperature ($2700\text{--}3000^\circ\text{C}$), and characterized by a homogeneous structure and a low surface area around $100 \text{ m}^2/\text{g}$, so they are often described as non-porous sorbents. Their higher efficiency over C_{18} silica for trapping polar pesticides has been shown, mainly by the group of Di Corcia et al. [124,160–170]. GCB cartridges have been successfully employed for the preconcentration of

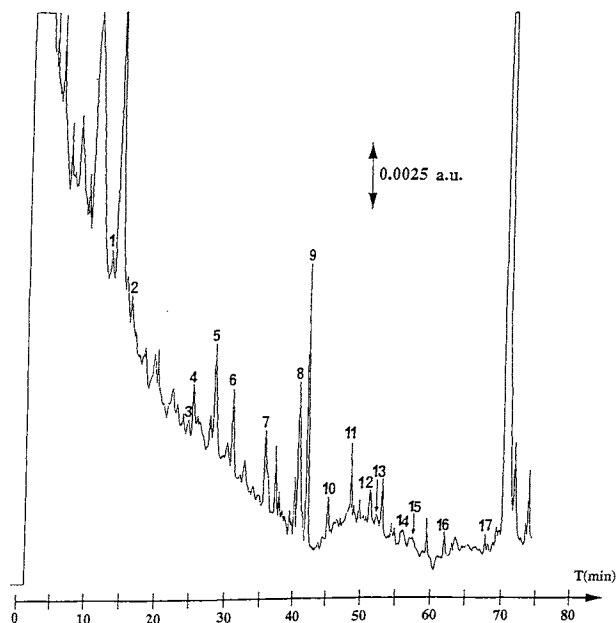


Fig. 4.19. Analysis of 500 ml of River Seine water spiked with $0.1 \mu\text{g/l}$ of herbicides at pH 7. See Fig. 4.18 for experimental conditions.

organic pollutants such as phenols, chloroanilines, aromatic hydrocarbons, and organochlorine insecticides, and the multiresidue analysis of pesticides [160–174]. In Table 4.13, the recoveries obtained from 2 l of water using a 1 g GCB cartridge are compared to those obtained using a 1 g C_{18} silica with the same sample volume and to those obtained by using LLE with three separate 120 ml portions of methylene chloride [170]. Of course, breakthrough has occurred for most of the pesticides with a sample volume far lower than 2 l on a 1 g C_{18} silica cartridge, but not on GCB even for polar degradation products and the polar carbamates methomyl and oxamyl. As with other carbonaceous sorbents made from carbon blacks, various functional groups are present at the surface of the carbons owing to oxygen chemisorption [175]. Taking advantage of the positively charged active centre on its surface, Di Corcia et al. presented a multiresidue method for pesticides in drinking water based on a complete separation of basic and neutral pesticides from acidic ones [161,169].

Graphitic carbon blacks are not sufficiently pressure resistant to be used in liquid chromatography so no data indicating the LC behaviour of solutes are available. Pyrocarbon-modified silicas [176] and pyro-modified carbon black [177] were synthesized by Colin et al. as LC stationary phases. In recent years, a porous graphitic carbon (PGC) has been available in SPE cartridges which has been derived from a carbon material especially made for LC [178] under the trade mark Hypercarb™, which appeared at the end of the 1980s. It is characterized by a highly homogeneous and ordered structure and by a specific area around $250 \text{ m}^2/\text{g}$. This sorbent shows a

TABLE 4.13

RECOVERY DATA OBTAINED BY USING VARIOUS TECHNIQUES TO EXTRACT PESTICIDES ADDED TO 2 l OF A MUNICIPAL WATER SAMPLE

Compounds	% Recovery \pm RSD		
	LLE	C ₁₈	GCB
Omethoate	58 \pm 8	3 \pm 45	83 \pm 6
Butocarboxim sulfoxide	13 \pm 14	3 \pm 42	102 \pm 5
Aldicarb sulfoxide	16 \pm 17	4 \pm 29	93 \pm 5
Butoxycarboxim	74 \pm 7	4 \pm 32	98 \pm 3
Aldicarb sulfone	58 \pm 11	6 \pm 21	75 \pm 8
Oxamyl	51 \pm 10	24 \pm 12	101 \pm 2
Methomyl	64 \pm 11	10 \pm 20	100 \pm 2
Monocrotophos	68 \pm 5	42 \pm 16	98 \pm 3
De-isopropylatrazine	87 \pm 4	15 \pm 14	102 \pm 4
Dicrotophos	78 \pm 8	83 \pm 10	98 \pm 3
Fenuron	60 \pm 8	19 \pm 12	99 \pm 3
Metamitron	79 \pm 4	28 \pm 12	95 \pm 5
Vamidithion	57 \pm 8	87 \pm 5	98 \pm 4
Isocarbamid	74 \pm 10	78 \pm 6	97 \pm 3
De-ethylatrazine	85 \pm 4	30 \pm 13	97 \pm 4
Chloridazon	75 \pm 4	31 \pm 11	100 \pm 3
Dimethoate	78 \pm 6	22 \pm 14	98 \pm 4
Mevinphos	79 \pm 7	92 \pm 6	94 \pm 3
Cymoxanil	89 \pm 9	28 \pm 11	94 \pm 4
Butocarboxim	82 \pm 5	63 \pm 9	95 \pm 4
Aldicarb	68 \pm 12	55 \pm 9	99 \pm 4
Metoxuron	83 \pm 5	101 \pm 3	97 \pm 3
Hexazinone	75 \pm 11	88 \pm 4	98 \pm 3
Bromacil	74 \pm 7	87 \pm 5	97 \pm 2
Phosphamidon	85 \pm 9	94 \pm 4	98 \pm 3
Metribuzin	79 \pm 7	70 \pm 7	96 \pm 3
Dichlorvos	64 \pm 8	64 \pm 9	85 \pm 10

LLE and SPE using a 1 g C₁₈ cartridge and a 1 g GCB cartridge (spike level 1–4 μ g/l; mean values from four determinations). Adapted from Ref. [170] with permission.

reversed-phase behaviour in the sense that the retention is reduced when the methanol content of the mobile phases increases. Owing to its crystalline structure, made of large graphitic sheets held together by weak Van der Waals forces, it is often presented as a more retentive reversed-phase sorbent than C₁₈ silica [179]. However, the retention mechanism has been shown to be very different from that observed on C₁₈ silicas or SDB polymers. Compounds are retained by both hydrophobic and electronic interactions, so that non-polar analytes, but also very polar and water-soluble analytes, have been shown to be retained in water [149,150,158,180–182]. As an example, the retention factor in water of the very polar 1,3,5-trihydroxybenzene

TABLE 4.14

RETENTION FACTORS OF VARIOUS PESTICIDES MEASURED IN PURE ORGANIC SOLVENTS (METHANOL, TETRAHYDROFURAN AND METHYLENE CHLORIDE) WITH A PGC ANALYTICAL COLUMN AND CALCULATED DESORPTION VOLUME, V_r , OF METHANOL USING A 500 mg PGC CARTRIDGE. (LOG P_{ow} VALUES FROM REF. [102]) (FROM REF. [184])

Compound	$\log P_{ow}$	$\log k'$			V_r (ml)
		MeOH	THF	CH_2Cl_2	MeOH
Oxamyl	-0.5	-0.51	-1.42	-1.22	2
Methomyl	0.2-1.8	0.04	-0.92	-1.01	3
Metamitron	0.8	>1.4	0.23	0.26	>34
Fenuron	0.5-1.2	0.28	-0.56	-0.66	4
DIA	1.1	0.57	-0.71	-0.28	6
DEA	1.5	0.22	-1.01	-1.04	3
Metoxuron	1.6	1.28	0.05	-0.02	26
Metribuzin	1.6-1.7	-0.35	-1.42	-1.04	2
Aminocarb	1.7	-0.25	-1.35	-1.01	2
Carbendazim	1.4-1.6	>1.4	0.79	n.d.	>34
Chloridazon	1.1-2.2	0.96	-0.13	-0.05	13
Simazine	1.5-2.3	0.97	-0.49	-0.39	13
Atrazine	2.2-2.8	0.62	-0.82	-0.85	7
Diuron	2.8	>1.4	0.17	n.d.	>34
Linuron	2.8	1.38	-0.10	-0.16	32
3,5-Dichlorophenol	3.6	0.52	-0.73	-0.51	6
2,4,5-Trichlorophenol	4.1	0.99	-0.12	0.20	14
Anthracene	4.7	>1.6	1.21	n.d.	>34
Pentachlorophenol	5	>1.4	0.81	n.d.	>34

n.d., not determined.

(phloroglucinol) is about 1000 with PGC whereas it was found to be 3 with PRP-1 [180]. This compound is not retained by C_{18} silica and it was even proposed as an experimental probe for determining the void volume of C_{18} columns [183].

Because the retention mechanism in the cases above is different, $\log k'_w$ cannot be predicted from the water-octanol partition coefficient, as can be seen in Fig. 4.16b. It is clear that there is no relationship between $\log k'_w$ and $\log P_{ow}$ except for alkylbenzenes. Although $\log k'_w$ can be calculated using the electronic distribution in the solute molecule, it is not as easy as for C_{18} silicas or apolar copolymers [158]. From a qualitative point of view, it was found that high retentions are obtained for planar molecules containing several polar groups with electronic charges delocalized via π -bonds and lone pairs of electrons. However, a rapid and easy measure is to inject the polar analyte of interest onto an available analytical column of PGC with a methanol-water mobile phase and to estimate $\log k'_w$ values via the relationship between $\log k'$ and the methanol content.

Desorption conditions. On PGC, a high retention has been observed for some sol-

utes with pure organic solvent as mobile phase, which illustrates how the desorption is not as straightforward as on C_{18} silica and SDB sorbents.

Desorption problems have been encountered with GCB cartridges; pure methanol, acetonitrile or methylene chloride were shown to be unable to desorb some pesticides as well as other organic pollutants, so that a mixture of methylene chloride-methanol (80:20, v/v) was recommended [124,161,170]. It was also pointed out that residual water had to be reduced to a minimum and, that, when this is not done, low and irreproducible recoveries were obtained because the water can hinder intimate contact between the desorption mixture and the GCB. Since only a fraction of the residual water can be removed by vacuum, the authors recommended that one should wash the GCB cartridge with a small volume of pure methanol before applying the methylene chloride-methanol mixture, but risks of loss may exist for weakly retained compounds. Special care also has to be taken with the elution mixture owing to the problem of double-layers during the subsequent evaporation if the water was not well removed before desorption. Another problem mentioned by the authors is in the removal of methylene chloride when the subsequent analysis is performed by LC and when the desorption mixture cannot be blown down to dryness because of the presence of relatively volatile compounds. Most of the pesticides reported in Table 4.13 were extracted with good recoveries from 2 l samples using a 300 mg GCB cartridge and 6 ml of the desorption mixture, except for the first seven compounds of the list for which a 1 g GCB cartridge was required. Then, the desorption volume has to be so strongly increased with 1 g cartridges that a backflush desorption was recommended [170]. Recently a reversible SPE cartridge packed with PGC was specially designed by Altech (Carbograph).

All the problems mentioned above should be overcome if a water-miscible solvent could be used for the desorption and if the retention behaviour of analytes with pure organic solvent were known since, according to the theoretical basis of SPE, the desorption conditions can be derived from LC data. This was recently achieved using PGC sorbents. The retention factors of some pesticides and other organic pollutants have been measured using a PGC analytical column eluted with methanol, THF and methylene chloride and are reported in Table 4.14. The results show first that the retention factor can be very high with methanol and that THF and methylene chloride are more eluting. Some measurements were also performed with acetonitrile, but the retention factors were similar to those obtained with methanol. Once more, one can observe that there is no relationship between polarity, -indicated by corresponding values of $\log P_{ow}$ - and the retention of compounds. A polar pesticide such as metamitron is highly retained in pure methanol, and less by THF and methylene chloride. As with the prediction of the breakthrough volume for the percolation step, we have shown that the desorption volume can be estimated from the retention volume V_r , although the V_m volume (see Fig. 4.6a) should be more appropriate [184]. Using a 500 mg PGC cartridge, the calculation of the desorption volume of atrazine by approximation to V_r gives 7 ml and complete calculation of V_m (assuming 10 plates in the cartridge and using the equations reported in Section 4.2.3.2.5) gives a

TABLE 4.15

RECOVERIES OBTAINED WHEN DESORPTION IS PERFORMED USING METHANOL IN THE SAME WAY (FORWARD) OR IN THE OPPOSITE (BACKFLUSH) WAY TO SAMPLE APPLICATION (FROM REF. [184])

Compound	% Recovery \pm RSD ^a		
	Test of volatility	Forward desorption (15 ml)	Backflush desorption (5 ml)
Oxamyl	100 \pm 3	91 \pm 8	101 \pm 5
Methomyl	95 \pm 3	94 \pm 6	99 \pm 4
De-isopropylatrazine	103 \pm 2	103 \pm 4	102 \pm 6
Monocrotophos	102 \pm 3	105 \pm 5	100 \pm 5
Fenuron	95 \pm 4	95 \pm 3	101 \pm 5
Metamitron	95 \pm 3	Non-desorbed	99 \pm 3
De-ethylatrazine	103 \pm 2	100 \pm 3	101 \pm 4
Chloridazon	105 \pm 2	Non-desorbed	106 \pm 5
Carbendazim	65 \pm 9	Non-desorbed	58 \pm 7
Aldicarb	71 \pm 3	80 \pm 5	79 \pm 6
Aminocarb	103 \pm 3	Non-desorbed	103 \pm 3
Metribuzin	102 \pm 3	Non-desorbed	101 \pm 6
Metoxuron	98 \pm 4	Non-desorbed	101 \pm 5

Extraction of pesticides using a 500 mg PGC cartridge from 1 l of LC-grade water; spike level, 3 μ g/l; values from three replicate experiments. The volatility test consisted in spiking 5 ml of methanol with 50–100 ng of each pesticide and in evaporating to dryness under the same conditions of nitrogen flow at ambient temperature.

volume of 12.3 ml. Experimental measurements indicated that a volume of methanol around 12–14 ml was required for a complete desorption. Table 4.14 clearly indicates that methanol (and acetonitrile) cannot be used for desorption. Although THF and methylene chloride are more eluting, some compounds such as metamitron, diuron and carbendazim are too strongly retained. THF gives lower retention than methylene chloride for the compounds of Table 4.14, but from LC data, more than 20 ml of THF should be necessary to completely desorb carbendazim.

Because it is not really straightforward to predict which compounds will be too strongly retained by THF or methylene chloride for desorption with a low volume, the recommended solution is to always desorb analytes in the opposite way to the sample application (backflush desorption) when using carbonaceous sorbents, whatever the amount of sorbent in the cartridge. This is, of course, more important with PGC than with GCB because we can predict a higher retention on PGC owing to the higher specific area. Then, methanol can be used with the advantage of being water-soluble and less toxic than THF or methylene chloride. Table 4.15 shows the recoveries that were obtained by percolating 1 l of water spiked with 13 pesticides through a 500 mg PGC cartridge and desorbing it in the same way as the sample application with 15 ml of methanol and in the opposite way with 5 ml of methanol. Half of the

TABLE 4.16

COMPARISON OF RETENTION FACTORS IN WATER ($\log k'_w$) OBTAINED FOR C₁₈ SILICA, PRP-1 AND PGC FOR SIMAZINE, ATRAZINE AND NINE DEGRADATION PRODUCTS (FROM REF. [182])

Compound	$\log P_{ow}$	$\log k'_w$ C ₁₈	$\log k'_w$ PRP-1	$\log k'_w$ PGC
Atrazine	2.7	3.4 ± 0.2	>4	>4
Simazine	2.3	3.0 ± 0.2	>4	>4
De-ethylatrazine	1.6	2.6 ± 0.1	3.5 ± 0.3	3.2 ± 0.2
Hydroxyatrazine	1.4	2.5 ± 0.1	3.0 ± 0.2	3.4 ± 0.2
De-isopropylatrazine	1.2	2.1 ± 0.1	3.1 ± 0.2	>3.5
Hydroxy-de-ethylatrazine	0.2	1.5 ± 0.1	1.8 ± 0.1	2.8 ± 0.2
De-ethylde-isopropylatrazine	0	1.3 ± 0.1	1.2 ± 0.1	2.8 ± 0.2
Hydroxy-de-isopropylatrazine	-0.1	1.0 ± 0.1	1.0 ± 0.1	3.0 ± 0.2
Cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine)	-0.2	<0.5	<0.5	2.6 ± 0.1
Ammelide (2-amino-4,6-dihydroxy-1,3,5-triazine)	-0.7	<0.5	<0.5	2.5 ± 0.1
Ammeline (2,4-diamino-6-hydroxy-1,3,5-triazine)	-1.2	<0.5	<0.5	2.4 ± 0.1

Experimental or extrapolated values from measurements in water-methanol mobile phases

solutes were not desorbed in the forward flush, and the results are in agreement with the LC data of Table 4.14 whereas all the compounds are desorbed in the backflush way with only 5 ml of methanol. The lower recoveries for aldicarb and carbendazim are explained by loss during the evaporation step. For these compounds, it is necessary to stop the evaporation before dryness.

Application to the extraction of some very polar degradation products of atrazine. LC data obtained with a PGC analytical column have been compared to LC data obtained with C₁₈ silicas and the PRP-1 apolar copolymer [149,150,158, 182]. The potential of PGC for extracting very polar pesticide metabolites is shown in Table 4.16 for nine dealkylated and hydroxylated degradation products of atrazine down to cyanuric acid [182]. Most of them have $\log P_{ow}$ values lower than unity, indicating that these solutes are more soluble in water than in organic solvents. The limitation of both C₁₈ silica and PRP-1 polymer is clearly shown for the very polar ammelide, ammeline and cyanuric acid, with $\log k'_w$ values lower than 0.5 whereas they are higher than 2 with PGC. Retention data have been measured by packing a small column with a HSA/SDB (1060 m²/g) available in cartridges, and $\log k'_w$ values of 2.3 ± 0.1 and 1.8 ± 0.1 have been obtained for hydroxy-de-ethylatrazine and hydroxy-de-isopropylatrazine, respectively, and the three more polar metabolites were just slightly retained [185]. Therefore, the new HAS/SDB sorbents cannot be used for the extraction of very polar analytes. Using a 300 mg PGC cartridge, recoveries were above 90% with the handling of 250 ml of water sample for all the metabolites except the three most polar ones for which a 500-mg cartridge was required to obtain similar recoveries. Figure 4.20a shows the chromatogram of a drinking water extract spiked with 0.5 µg/l of si-

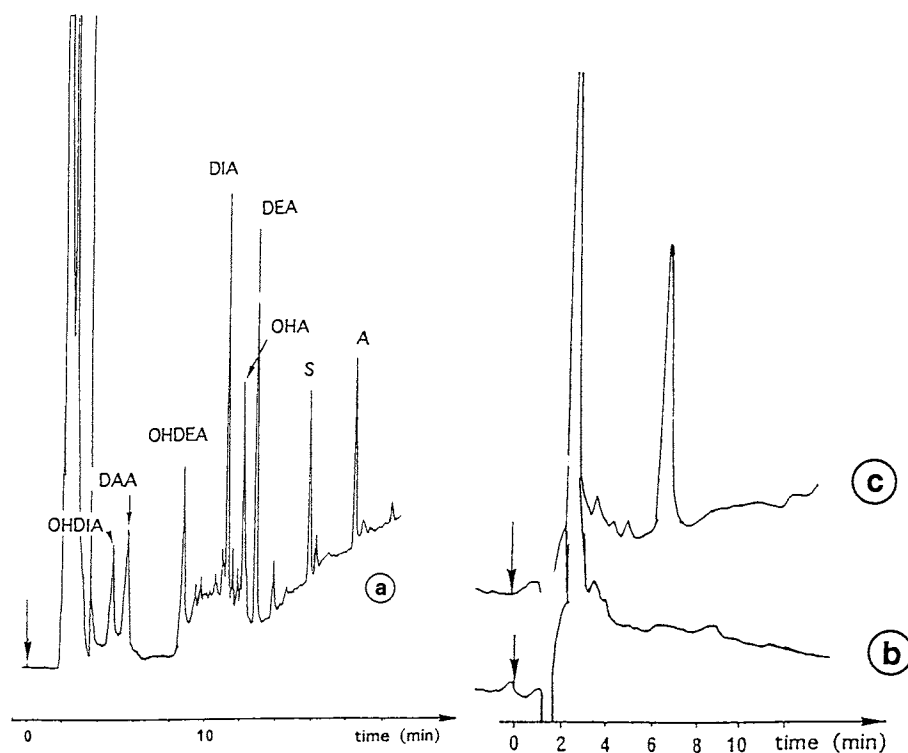


Fig. 4.20. (a) Off-line analysis of the extract corresponding to 250 ml of drinking water spiked with $0.5 \mu\text{g/l}$ of atrazine, simazine and six hydroxylated and dealkylated metabolites. (b) Analysis of the extract corresponding to 250 ml of drinking water non-spiked and (c) spiked with $5 \mu\text{g/l}$ of cyanuric acid. From Ref. [182]. (a) Extraction through a 300 mg PGC cartridge, and analysis with a Spherisorb ODS-2 ($25 \text{ cm} \times 0.46 \text{ cm i.d.}$) analytical column; water–acetonitrile gradient at pH 7. Solutes: A, atrazine; S, simazine; DEA, de-ethylatrazine; OHA, hydroxyatrazine; DIA, de-isopropylatrazine; OHDEA, hydroxy-de-ethylatrazine; DAA, de-ethylde-isopropylatrazine; OHDA, hydroxy-de-isopropylatrazine; UV detection at 210 nm. (b) Extraction through a 500 mg PGC cartridge and analysis of the whole extract with a Hypercarb ($10 \times 0.46 \text{ cm i.d.}$) analytical column, mobile phase methanol–0.05 M sodium phosphate buffer at pH 7 (30/70, v/v); UV detection at 220 nm.

mazine, atrazine and six metabolites, and Fig. 4.20b shows the chromatogram corresponding to an extract of drinking water spiked with $5 \mu\text{g/l}$ of cyanuric acid. GCB have also been used for the extraction of the de-ethylde-isopropylatrazine from water before GC/MS determination [186].

4.2.3.3.4. Ion-exchangers. Ionic or ionizable organic compounds can be preconcentrated by ion-exchange sorbents. Silica-based ion-exchangers are mainly found in disposable SPE cartridges, with the inherent disadvantage of being limited to the pH range 3–9, but polymer-based ion-exchangers can also be found. They have the advantage of being utilized over the pH range 1–13 and also have a higher capacity than silica-based

ion exchangers. The retention mechanism is such that retention occurs at a convenient pH for solutes to be ionized. Desorption is performed at a different pH value, at which compounds are in their molecular form. If the compounds are ionic over the whole pH range, then desorption occurs by using a solution of appropriate ionic strength. The main problem encountered comes from the fact that environmental waters contain high amounts of inorganic ions which overload the capacity of these sorbents. A chemical sample pretreatment based on precipitation of calcium with oxalic acid and complexation of iron with EDTA can be carried out, but it has been used only in an on-line procedure [187–189]. This method was applied to the preconcentration of the pesticide aminotriazole which is polar and water-soluble, and not retained on C_{18} silica or polymers [190,191]. Since it is ionizable, a sulfonic acid-type of resin-based cation-exchanger was used. The breakthrough volume on a precolumn (10×2 mm i.d.) was measured as 150 ± 10 ml with LC-grade water spiked with aminotriazole. With drinking water samples, the breakthrough volume was below 5 ml. After the chemical pretreatment to remove inorganic anions, the recovery with a 30 ml sample of spiked drinking water was 18%. These results show that for very polar ionic species there is a competition between the remaining trace inorganic ions and organic ions, in favour of the inorganic ions. When the organic ions of interest are more hydrophobic, then additional interactions occur with the matrix of the ion-exchanger sorbent, so that the competition is in favour of the organic ions. One example is in the direct concentration of triazines at low pH using cation-exchanger cartridges.

For compounds which are ionizable in the range of pH 1–13, the above problem can be overcome by using a two-trap system. The basis of this system is that compounds are retained on apolar sorbents when they are in their molecular form, but not

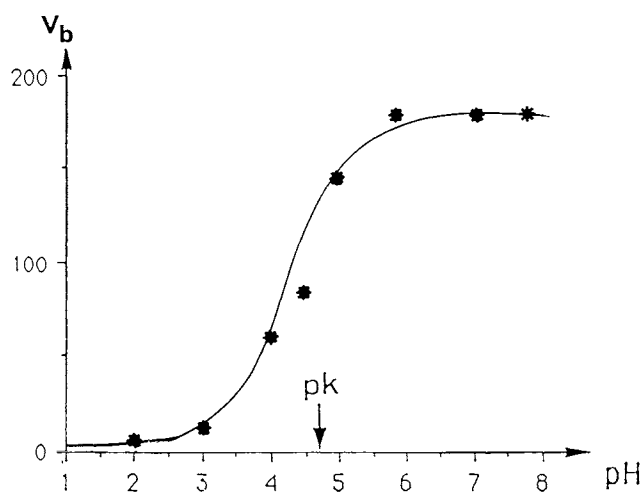


Fig. 4.21. Variation of the breakthrough volume of aniline with the pH of the aqueous sample percolated through a 9×0.46 cm column.

when they are in their ionic form. Figure 4.21 shows the dependence of the breakthrough volume of aniline ($pK_a = 4.8$) with the pH of the sample when using a column packed with the SDB PRP-1 sorbent [192]. The breakthrough volume reduces from 180 ml when aniline is in its molecular form to 6 ml when it is ionized. This curve shows the necessity of adjusting the sample to two units below or above the pK_a value to be sure of having the compounds in their ionized or molecular form. In the tandem system, the compounds are first trapped in their molecular form onto non-polar and non-specific sorbents. Then, they are desorbed from the sorbent by applying a few millilitres of deionized solution at pH adjusted in order to have solutes in their ionic form. If a second cartridge packed with an ion-exchanger is coupled in tandem to the first one, ionic solutes can be re-preconcentrated. This two-step extraction is selective since only ionic species are transferred. Although this methodology is often carried out with on-line techniques (see Chapter 5), in off-line techniques it has been successfully applied to the trace analysis of phenols, triazines and chloroanilines by using a GCB cartridge for the first trapping of solutes and by connection to a second cartridge packed with an appropriate ion-exchanger cartridges [164,168,170].

Mixed mechanisms are often observed with direct percolation of natural water samples through ion-exchangers, depending on the bonding procedure. This occurs with propylbenzenesulfonic acid cation exchangers, in which the retention of analytes is by both hydrophobic and ion-exchange interactions, with the advantage of reducing the competition with inorganic ions in natural waters. These sorbents were successfully used for the extraction of hydroxylated atrazine degradation products [193]. A SDB-based mixed-mode resin that contains C_{18} and sulfonated cation-exchange groups was also shown to be efficient for isolating triazines and basic drugs, combining hydrogen bonding, cation exchange, and Van der Waals interactions [194,195]. Recently, Empore disks of SDB-RPS have been commercially available with a sulfonated SDB resin embedded. These disks were shown to considerably enhance the recoveries of polar compounds such as catechol and phenol [196]. GCB has also been shown to be a reversed phase sorbent and an anion-exchanger owing to positively charged active centres such as benzyropyrium groups on its surface [169]. Therefore, it was shown that sufficiently acidic pesticides are strongly adsorbed on these active sites and could not be desorbed by conventional organic solvents. After sample-application through the GCB cartridge and removal of the residual water, a stepwise elution system allows the desorption of basic and neutral pesticides with a methylene chloride-methanol mixture (80:20, v/v) and subsequently the desorption of the acidic ones using the same composition of desorption mixture basified with 0.016 M potassium hydroxide [161]. No active sites have been shown so far on PGC sorbents.

4.2.3.3.5. Immunoaffinity sorbents. Apart from ion-exchangers, which are difficult to utilize with natural waters, the other sorbents which have been described above are non-selective. Although we have shown that their use can permit the determination of many pesticides at the $0.1 \mu\text{g/l}$ level in drinking water, we have also pointed

out that their non-selectivity is a major drawback for trace analysis in surface waters which can be achieved only with an additional clean-up. Even with clean-up step, it is still often difficult to exploit the chromatograms when the usual detectors are used, owing to many interfering peaks or noisy base-lines caused by the numerous unresolved analytes which are present at trace-level in the sample matrix.

Immunosorbents (IS) are obtained by bonding antibodies onto a sorbent, and their main feature is their high selectivity resulting from the antigen–antibody interaction. The use of IS for clean-up of biological samples is well known, but it is used with very few target analytes or metabolites [197–199]. In the environmental field, immunoaffinity cartridges are available for the clean-up of food extracts for the determination of mycotoxins [200–205]. However, owing to the recent increase of immunoassays in pesticide analysis, many antibodies against pesticides have been developed. The use of immunosorbents for the SPE of some target pesticides such as carbofuran, carbendazim, chlortoluron and atrazine has been described [206–210].

However, so many pesticides are applied that it seems difficult to develop IS for each one. Another approach described recently has the aim of providing IS which can extract only a group of pesticides from the matrix. Extraction, concentration and clean-up are combined in one step for pesticides within a chemical group using sorbents bound with specific antibodies which recognize several compounds with related structures. The technique takes advantage of both the high affinity of antibodies, allowing an efficient concentration, and the selectivity of their interaction that enables selective extraction of a whole chemical class of pesticides. Such immunosorbents have been made for the extraction and concentration of the groups of triazines and of phenylureas [211–213]. Polyclonal antibodies were specially made against isoproturon and chlortoluron. An IS made with the anti-isoproturon antibodies was shown to extract nine phenylureas in a mixture of 15 whereas an IS made with the anti-chlortoluron antibodies was able to extract all the phenylureas except fenuron. For the triazine groups, an IS made with anti-atrazine antibodies was able to extract most of the triazines. By mixing anti-simazine and anti-atrazine antibodies it is also possible to trap the hydroxylated and dealkylated degradation products [213]. Cartridges packed with silica-based IS are as easy to use as C_{18} silica cartridges, with activation, percolation of the samples, and desorption with a few ml of methanol–water mixture (70:30, v/v). A convincing illustration of the high selectivity provided by IS is shown in Fig. 4.22 which shows the chromatograms corresponding to the extraction of 200 ml of drinking water (Fig. 4.22a) and surface water (Fig. 4.22b) spiked with $0.5 \mu\text{g/l}$ of a mixture containing 13 phenylureas though a cartridge containing 1 g of IS anti-isoproturon [212]. The chromatogram in Fig. 4.22b looks like that obtained with the spiked drinking water sample with a similar baseline and very few interfering peaks. The development of these new extraction sorbents will improve the whole analytical scheme for pesticide analysis. The drastic reduction of interferences by matrix constituents allows a more reliable identification of pesticides at lower detection levels. As can be seen in Fig. 4.22c, in the chromatogram corresponding to the

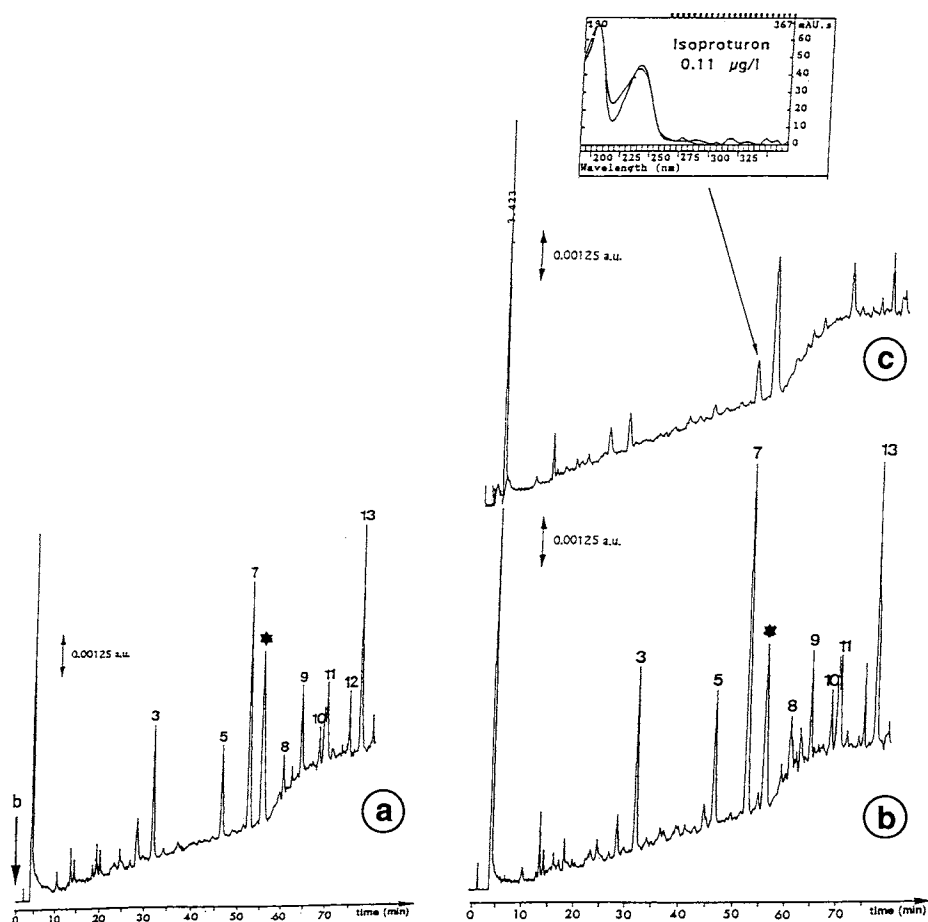


Fig. 4.22. Analysis of (a) a 200 ml drinking water sample extract and (b) a 200 ml surface water sample extract spiked with 13 phenylureas after extraction through a 1 g cartridge packed with an immunosorbent anti-isoproturon; (c) a 200 ml non-spiked surface water extract. Desorption with 4 ml of methanol-water, (70:30, v/v). Analytical column, SupelcosilTM LC-18-DB (25 × 0.46 cm), water-acetonitrile gradient. Solutes: (3) monuron; (5) chlortoluron; (7) isoproturon; (8) difenoxuron; (9) buturon; (10) linuron; (11) chlorbromuron; (12) diflubenuron; (13) neburon. From Ref. [212] with permission.

non-spiked surface water sample, isoproturon was easily identified at a concentration of 0.11 µg/l. The selectivity of the preconcentration is so high that the sample volume could be reduced to 50 ml. This was shown by spiking a 50 ml surface water sample with 0.1 µg/l of six triazines, using a 0.5 g cartridge packed with an anti-atrazine IS. Detection limits were in the 0.03 µg/l range for these triazines in surface water [213]. These immunosorbents have also been shown to be very efficient for the clean-up of plant tissues and food matrices prior to the determination of phenylureas and triazines [214,215].

4.2.3.4. Conclusion

In this section, emphasis has been given to the theoretical basis of SPE, in order to help in the selection of both the sorbent and the sample volume, the key parameters of this method. The different sorbents have been reviewed, and it is clear that development of the selective sorbents is an active area of research.

However, SPE is not completely free from practical problems such as the risk of overloading the column by percolating unknown samples with high content of contaminants, or of early breakthrough caused by a blocking of the pores. Competitive processes exist between the compounds of interest and the components of the sample matrix.

Nevertheless, the advantages over LLE are more numerous :

- simplicity;
- speed, and possibility of predicting the experimental parameters (sample, volume, sorbents);
- sampling in the field, avoiding transport of voluminous samples, and allowing good storage;
- efficiency: no formation of emulsions, purer samples;
- safety: the use and disposal of flammable solvents and exposure of chemists to toxic solvents are reduced to a large extent;
- low cost : less labour, solvent and transport;
- easy automation, and the possibility of on-line coupling with the separation step.

4.2.4. Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) is a new, fast and simple analytical technique which uses coated fused-silica fibres to extract analytes from aqueous samples. The subsequent analyses are currently performed by GC analysis and the analytes are desorbed in the injector of the gas chromatograph. This method was introduced by Pawliszyn and his group, some 6 years ago, and represents a further advance as a solvent-free alternative to the extraction of organic compounds from water samples [216–221]. The technique was first applied to the trace determination of volatile organic compounds such as substituted benzenes, toluenes and xylenes (BTX) and chlorinated hydrocarbons [216,222–224]. Then, with the availability of new fibres, various semi-volatile groups of compounds such as phenols, polycyclic aromatic hydrocarbons and polychlorinated biphenyls were successfully extracted from aqueous samples [225–229]. More recently, the method was applied to other environmental pollutants such as organophosphorus pesticides, nitrogen containing pesticides, triazine- and 2,6-dinitroaniline pesticides and metolachlor [230–236].

4.2.4.1. Description and set-up

An outstanding feature of the SPME sample-preparation method is its simplicity.

Organic pollutants are adsorbed from aqueous (or gaseous) samples by the solid phase coating of a silica fibre support. The analytes are then directly transferred to the injector of a gas chromatograph using a modified syringe assembly where they are thermally desorbed and analyzed. In contrast to conventional extraction methods, the total amount of extracted sample is used for the determination by GC. The SPME device uses a conventional syringe (from Hamilton) which makes the system portable and is shown in Fig. 4.23. The bottom centimetre of the fibre is coated with stationary phase. The fibre is glued into a length of stainless steel rod with high temperature epoxy resin which runs up through the syringe needle. The rod is inserted into the syringe and glued to the top of the plunger. When the plunger is retracted, the fibre is drawn into the needle and this protects the fibre when the needle is used to pierce the septum of a sample vial or the GC injector. The plunger can then be pushed down to expose the fibre to the sample or to the GC carrier gas during the thermal desorption [228]. The device is available from Supelco and comprises a reusable syringe assembly, and a replaceable fibre assembly. It can be used in a manual procedure or be included in an automated Varian autosampler. Polydimethylsiloxane and polyacrylate phase-coated fibres are now available in various thickness (7, 30 and 100 μm for polydimethylsiloxane fibre assemblies, and 85 μm for polyacrylate).

The miniature cylindrical geometry of this apparatus permits rapid mass transfer during extraction and then desorption of the concentrated extract into the GC. In the first step, the coated fibre is exposed to the sample or its headspace and the target analytes partition from the sample matrix into the coating. So far, the fibre with the concentrated analytes is then transferred to a GC where compounds are thermally desorbed, separated and quantified. No special thermal desorption module and no modification of the GC is needed. Septum programmable, split-splitless and on-column injectors have all been used for SPME desorption. Organic solvents are completely eliminated in this procedure. The aqueous sample volumes are typically 1–5 ml.

4.2.4.2. Basic parameters

In a finite volume of sample, V_{aq} , the number of moles, n_{s} , of analyte adsorbed, by the fibre at equilibrium is dependent on the initial analyte's concentration in solution, C_{aq} , according to

$$n_{\text{s}} = \frac{KV_{\text{s}}V_{\text{aq}}C_{\text{aq}}}{KV_{\text{s}} + V_{\text{aq}}}$$

where K is the partition coefficient of the analyte between the stationary phase and the aqueous phase, and V_{s} the volume of polymeric stationary phase. The dynamics of such a model has been mathematically modelled for both stirred and unstirred solutions, by equations for the diffusive and connective transport of analytes in the

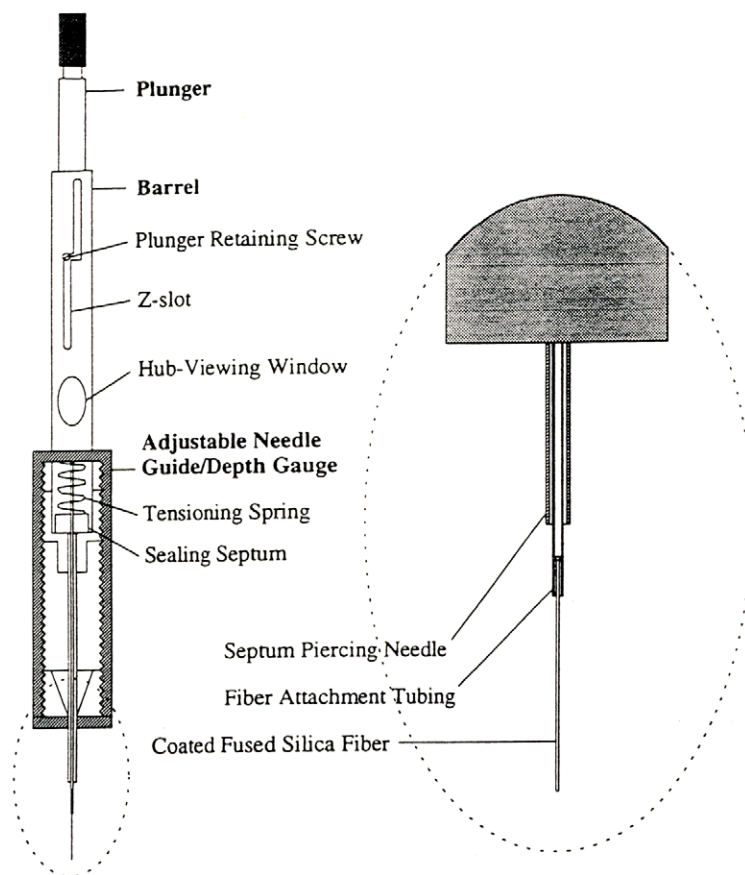


Fig. 4.23. Schematic of the SPME device. From Ref. [228].

sample matrix and the extractive phase [218]. The model predicts that rapid extractions in less than 1 min are possible if the solution is completely mixed as represented in Fig. 4.24 [228]. Without mixing, the time required to reach equilibrium is limited by diffusion in water. With inefficient mixing, an unstirred layer of water remains next to the fibre, which limits the rate of sorption because the analytes must first diffuse across this static layer. In practice, stirring with a magnetic bar fails to provide perfect mixing, so the static water layer increases equilibration times to a few minutes [217]. Therefore, extractions are usually performed under stirred conditions. It can also be seen from the above equation that the mass adsorbed and the linear range depend on the partition coefficient and the volume of stationary phase. The choice of the stationary phase is therefore important. The equilibration time depends on the partition coefficient. The higher the K value, the larger is the amount extracted at equilibrium. The number of phases now available

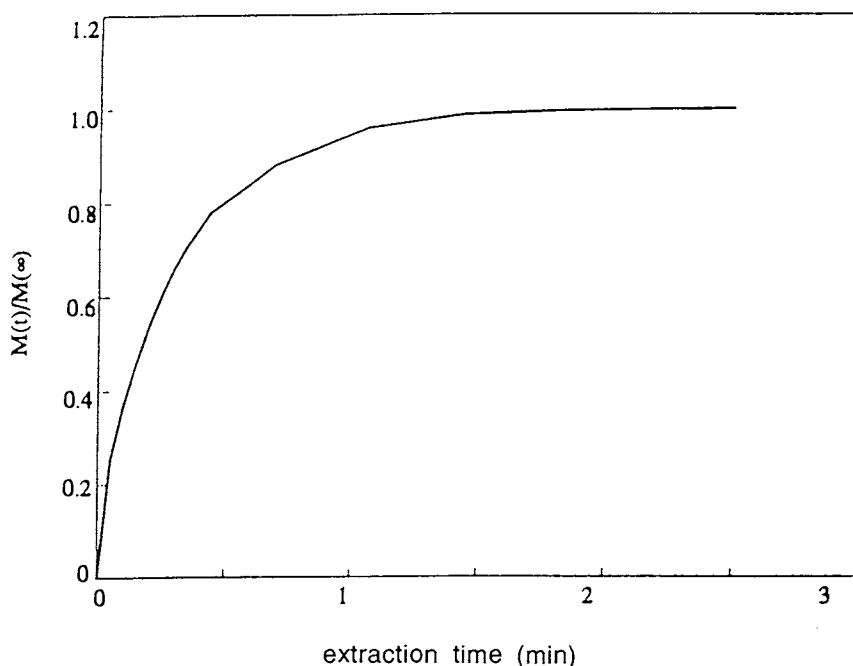


Fig. 4.24. Theoretical extraction-time profile when diffusion in the coating determines the extraction rate. From Ref. [228].

is limited, but the situation should change because of the rapid developments in this area.

In practice, the headspace technique is preferred for volatile analytes, especially in dirty matrices. For semi- and rather non-volatile compounds, as are most of the analyzed GC-amenable pesticides, the fibre is generally immersed in the aqueous samples. Optimization of the procedure consists of (i) selecting the phase type and the thickness of the phase, (ii) optimizing desorption times and temperatures, (iii) optimization of the sorption process by generating time profiles for each analyte in order to select the exposure time of the fibre under stirring conditions, and (iv) testing the linearity of the method.

The selection of the fibre is guided by the nature of the analytes; the non-polar polydimethoxysilane fibre is preferred for the extraction of non-polar pesticides with very low solubility in water such as organochlorine pesticides and some of the non-polar organophosphorus compounds whereas the more polar polyacrylate fibre was shown to be more appropriate for the more polar nitrogen-containing herbicides. As can be seen below, a shorter time than the equilibrium times can be selected but this may affect the sensitivity and precision of the method. In such conditions it is important that the extraction time should be monitored carefully, because when equilibrium is not established, slight deviations in the extraction times may result in devia-

tions in the amounts extracted. Other parameters can also be studied, such as the modification of the ionic strength, or adjustment of pH. This is particularly important with the extraction of water-soluble compounds, because the more soluble the analyte is in the water, the lower is the affinity of that analyte for the fibre coating. Therefore, the amount of analyte extracted by the fibre can be increased by reducing the solubility of the analyte, which can be achieved by the addition of salt or by pH adjustment.

The various steps in the optimization, and the effect of some parameters, are given in some applications below.

4.2.4.3. *Effects of the various parameters of interest, with selected applications*

4.2.4.3.1. *Desorption conditions.* Optimal desorption conditions are determined by trying various temperatures for different lengths of time. The time and temperature required to successfully desorb organochlorine pesticides from a 100- μm polydimethylsiloxane fibre were 2 min at 275°C [230]. For nitrogen-containing pesticides, desorption from a 95- μm polyacrylate was achieved within 5 min at 230°C [229]. Desorption of metolachlor was performed at 200°C for 2 min using a 100- μm polydimethylsiloxane fibre [232]. Obviously, desorption is not difficult and the polyacrylate phase can be heated up to 300°C.

4.2.4.3.2. *Time profiles, equilibrium times, and partition coefficients.* Equilibration times are usually determined from time profiles, which consists in reporting on a graph the peak areas obtained, or amount extracted, when varying the extraction time from a few minutes to 120–180 min. In contrast to the SPME of volatile analytes, equilibrium times were of the order 15–180 min for organochlorinated pesticides using a polydimethylsiloxane fibre and for various nitrogen-containing pesticides using a polyacrylate fibre [229–231]. Examples of experimental time profile curves are given in Fig. 4.25a–d for four organophosphorus pesticides using a 85- μm polyacrylate (Fig. 4.25a) and a 100- μm polydimethylsiloxane (Fig. 4.25b) fibre and for four triazines (Fig. 4.25c) and four 2,6-dinitroaniline herbicides using a 85- μm polyacrylate fibre [231]. First, with the polyacrylate fibre, equilibrium was not achieved in 2 h for the organophosphorus compounds and 2,6-dinitroaniline whereas it is reached after that time for triazines. Equilibrium is reached more rapidly for the organophosphorus pesticides by using the polydimethylsiloxane fibre than with the polyacrylate one. The effect of the fibre used for the organophosphorus pesticides can be seen by comparison of Fig. 4.25a,b. The equilibrium time is shorter with the polydimethylsiloxane fibre and, if the amounts extracted are similar, the affinities are not the same. Since the GC separation was achieved in 25 min, an exposure time of 25 min was found to be a reasonable compromise for a good peak response and acceptable time. Therefore, during the GC run, the following sample is extracted. When comparing the peak responses of the standard solution with those after SPME,

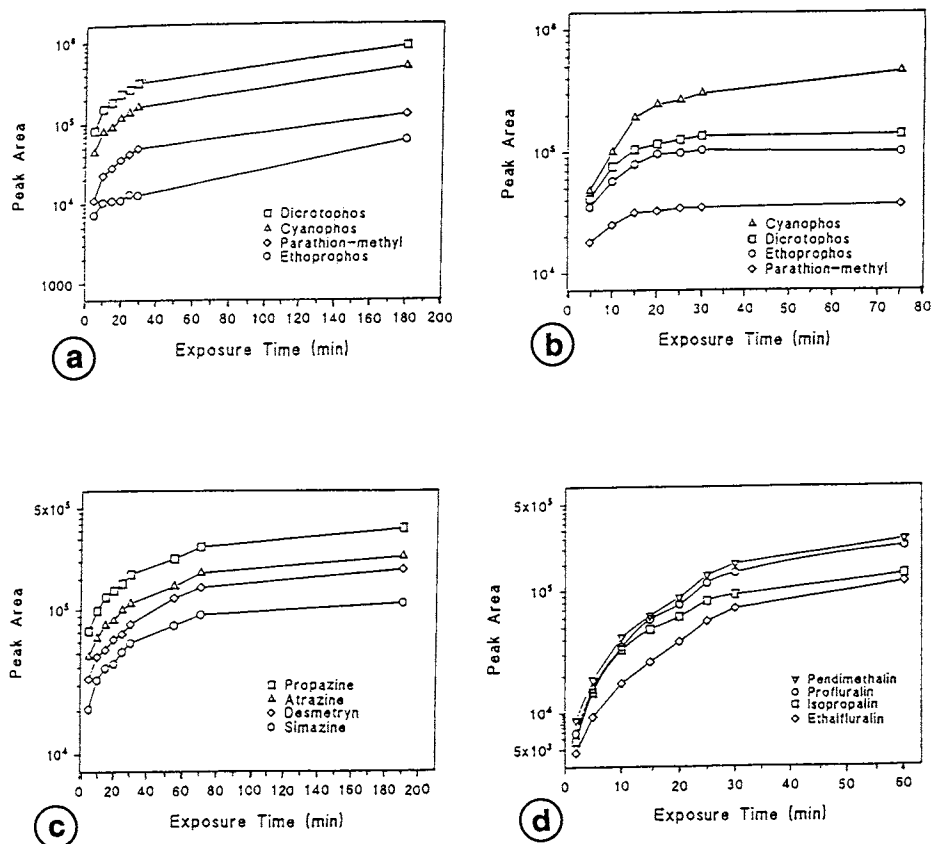


Fig. 4.25. Time-dependence of the equilibration of four organophosphorus (a,b), four triazine (c) and four 2,6-dinitroaniline herbicides (d) between the aqueous and the polyacrylate phase (a,c,d) and polydimethylsiloxane phase (b). From Ref. [231] with permission.

there was a pronounced reduction in peak response after SPME, even for chemically related compounds such as triazines. For related pesticides it was shown that the peak response was quantitatively related to the hydrophobicity of the analytes, as expressed by $\log P_{ow}$, as shown in Fig. 4.26. It seems clear that the equilibration of the analytes depends on the hydrophobicity of the compounds and that the more hydrophobic, or less polar, compounds are more readily absorbed by the polyacrylate phase.

From time profiles, Boyd-Boland and Pawliszyn [229] found similar experimental equilibrium times for 22 nitrogen-containing herbicides using a 95- μm polyacrylate fibre, as shown in Table 4.17. Equilibrium times ranged from 10 to 120 min with more than half of the analytes reaching equilibrium by 50 min. The time of extraction was selected at 50 ± 2 min. The effect of selecting lower extraction times than

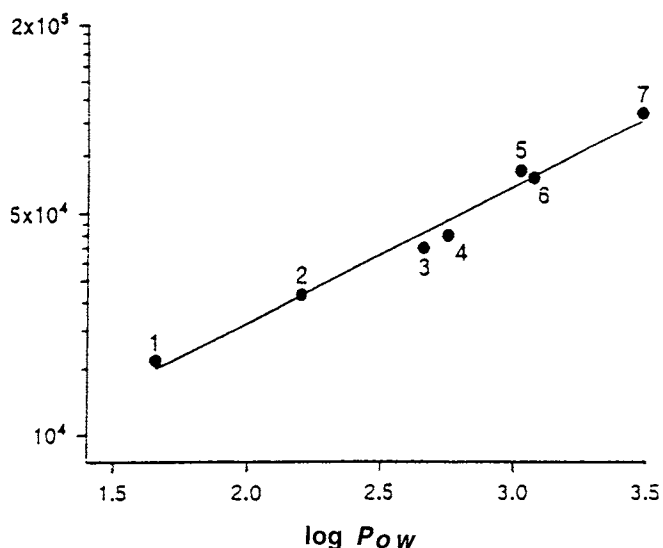


Fig. 4.26. Dependence of peak response (extraction efficiency) on octanol–water partitioning coefficient P_{ow} (double logarithmic scale). Assignment: (1) cyanazine; (2) simazine; (3) simetryn; (4) atrazine; (5) propazine; (6) ametryn; (7) prometryn. From Ref. [231] with permission.

equilibration times will affect more the precision and sensitivity of compounds with small K values and with equilibrium times less than 50 min. Fortunately the analytes with small K values have equilibrium times less than 50 min. The results for K values indicate that there is a correlation with the solubility in water; the more soluble an analyte is, the lower the K value. Therefore, by reducing the solubility of a given analyte in water, the amount extracted can be increased. The effect of varying the ionic strength was tested for the compounds in Table 4.17, with three concentrations of NaCl (2 M, 4 M and supersaturated) and an increase was observed except for the nitroanilines, oxyfluorfen and oxadiazon. No significant effect of the pH was observed between 4 and 11: however, at pH 2 the extraction of dinitroanilines and oxyfluorfen was enhanced. Eisert and Levsen [231] also observed an increase in the amount extracted when NaCl was added to the sample, as shown in Fig. 4.27. While for the hydrophobic compounds an optimum extraction is reached at about 60% saturated sodium chloride solution, such a maximum is not observed with less hydrophobic compounds. The same authors have also studied the effect on peak response of adding methanol. An increase in the methanol content, up to 20% by volume, reduced the peak of terbutylazine by a factor of about 2 and that of ametryn by a factor of about 1.5. These results indicate that the effect of adding polar organics is not as important as could have been expected. This is an interesting result since the addition of a small amount of organic solvent in the samples can reduce the adsorption effect on glass and tubing.

TABLE 4.17

SELECTED NITROGEN-CONTAINING PESTICIDES. WATER-SOLUBILITY. EQUILIBRATION TIMES AND PARTITION COEFFICIENT BETWEEN A POLYACRYLATE FIBRE AND WATER. AND PRECISION OBTAINED FROM 50 min EXTRACTION

Herbicide		Data from time profiles ^a		Data from 50-min extractions and precision ^b		
Class name	Solubility in water (mg/l)	Equilibration times (min)	K value	Amount extracted (ng)	%RSD NPD	%RSD MS
<i>Triazines</i>						
Atrazine	70	90	2000*	1.1	14	9
Hexazinone	330000	10	300*	0.34	22	4
Propazine	8.6	90–120	3000*	0.7	12	14
Metribuzin	1200	50–90	200*	0.52	9	13
Simazine	3.5	10	300*	0.42	9	5
<i>Nitroanilines</i>						
Benfluralin	<1	50–90	7000	1.6	13	11
Isopropalin	0.1	50	5000	2.8	20	21
Pendimethalin	0.3	50	20000	2.5	14	17
Profluralin	0.1	30	7000	1.5	7	7
Trifluralin	20	50	8000	5	13	16
<i>Substituted Uracils</i>						
Bromacil	815	30	400*	0.58	22	10
Terbacil	710	50–90	200*	0.54	10	13
<i>Thiocarbamates</i>						
EPTC	365	90	4000*	1.9	9	10
Molinate	800	50–90	2000*	1.7	7	12
Cycloate	85	90	7000*	4.4	12	14
Butylate	45	90	3000*	4.1	13	13
Pebulate	60	90	4000*	12	12	13
Vernolate	107	90	10000*	1.5	12	12
<i>Others</i>						
Metolachlor	530	50	4000*	2.8	10	15
Oxyfluorfen	0.1	30	3000	7	14	14
Oxadiazon	0.7	50	20000	4	18	22
Propachlor	700	50	1000*	1.2	9	5

^aK values determined under optimum conditions; overnight extraction. 4M NaCl (*) or unsalted.

^bAverage of seven replicates, containing 10 ng/ml of each analyte. From Ref. [229].

4.2.4.3.3. *Precision of the method, linearity range and detection limits.* In Table 4.17 the precision of the method was given for a set of seven replicates using both the MS

and NPD detector. Differences in the precision achieved with the two detector systems were primarily due to the difficulty in accurately integrating small broad peaks on the NPD. However the majority of analytes were extracted with precision ranging from 2 to 20%. Eisert and Levsen reported precisions lower than 12% for a set of 34 nitrogen-containing pesticides from three replicate experiments at a spike level of 30 ng/ml with an extraction time of 25 min and a sample volume of 3 ml [231]. For organochlorinated pesticides, a precision in the range 2–20% was also observed with solutions spiked at 10 $\mu\text{g/l}$, using GC-FID or GC-MS, with an extraction time of 90 min using a polydimethylsiloxane fibre. A better precision was obtained for analysis performed on the ECD with a solution spiked at the 1 $\mu\text{g/l}$ level, due to its selectivity.

The reproducibility between fibres was found to be good. With eight extractions from aqueous standards using three different fibres, the percent difference for each comparison between fibres was less than 5% [232]. Each fibre can be re-used many times. One study reported a re-usability more than 100 times with distilled water and indicated that the fibre had to be replaced after 27 analyses in runoff water [232].

The linearity of the method has been investigated. With GC-NPD detection the linearity of the calibration curves was observed in the range 0.03–30 ng/ml for 14 organophosphorus compounds, 12 triazines and 8 2,6-dinitroaniline pesticides ($r = 0.996$ for most of the compounds) [231]. For 22 nitrogen-containing pesticides, the linearity was obtained over the range 0.1–1000 ng/ml with the GC-MS, GC-NPD and GC-MS and 21 had correlation coefficients greater than 0.99. With a detection limit below 2 ng/l (2 ppt) for metolachlor with GC-ECD, linearity was obtained over seven orders of magnitude up to 20 mg/l (20 ppm) [232].

Detection limits depend on the detection mode and on the analytes. It varied from 0.005–0.09 $\mu\text{g/l}$ for organophosphorus compounds, triazines and 2,6-dinitroaniline pesticides with GC-NPD [231]. Detection limits of nitrogen-containing pesticides, reported in Table 4.17, were in the range 0.2–20 $\mu\text{g/l}$ using GC-FID, 0.01–0.8 ng/l with GC-NPD, and much lower - in the range 0.01–15 ng/l with GC-MS. For organochlorine pesticides, the lowest detection limits were obtained with GC-ECD, in the range 0.05–9 ng/l whereas they were in the range 0.02–800 ng/l with GC-MS.

4.2.4.3.4. Effect of the sample matrix. The effect of the sample matrix on the only SPME step is difficult to estimate because it is usually combined with the selectivity of the GC detector. Moreover, since the methodology is rather recent, few applications have been reported for pesticide analyses in various natural samples. Figure 4.28 shows the chromatogram of a water sample from a ground water well (Germany) which has been contaminated by triazines as indicated by previous on-line SPE-LC-MS analysis performed by the same authors. The SPME coupled with GC-NPD analysis revealed the presence of ametryn, atrazine, propazine, simazine and simetryn in concentrations ranging from 0.05 to 0.3 $\mu\text{g/l}$ [231]. It is evident from

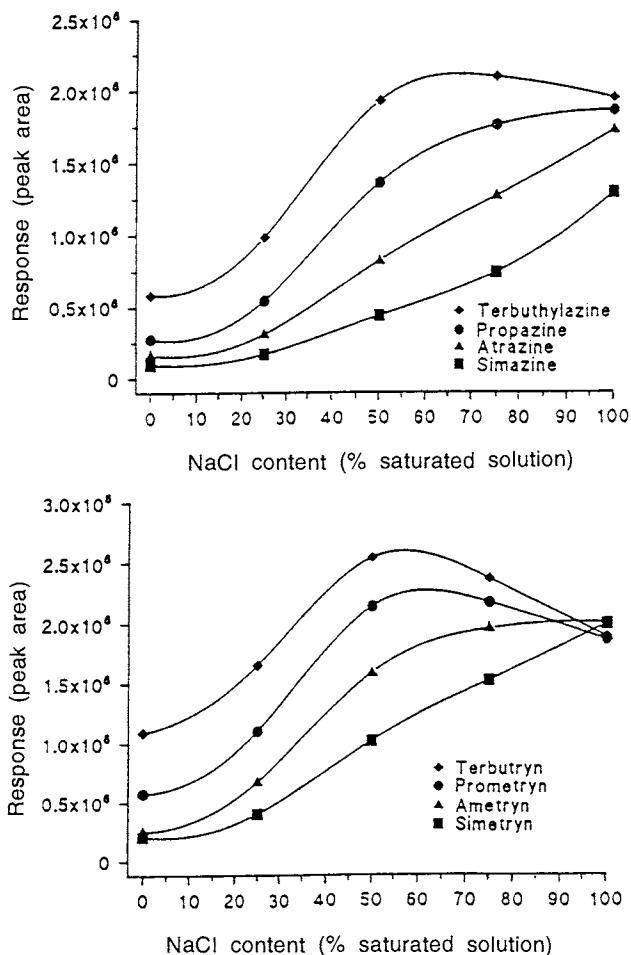


Fig. 4.27. (a,b) Dependence of peak response (extraction efficiency) on sodium chloride content between 0 and 100% saturated solution. From Ref. [231] with permission.

the chromatogram that there are several other unidentified peaks, but it should be easy to confirm these peaks by GC-MS. The authors have also studied river water samples and observed that humic substances had no significant influence on the extraction using the polyacrylate fibre.

The combination of SPME and a subsequent GC-ECD analysis provides clean chromatograms. Figure 4.29a shows the gas chromatogram obtained from a standard water sample spiked with 1 $\mu\text{g/l}$ of each organochlorine pesticide, and Fig. 4.29b the analysis of a natural surface water sample under the same analytical conditions for the SPME and GC-ECD analysis [230]. Organochlorine pesticides are found in low concentration, but, once again, need to be confirmed by using another GC-column or

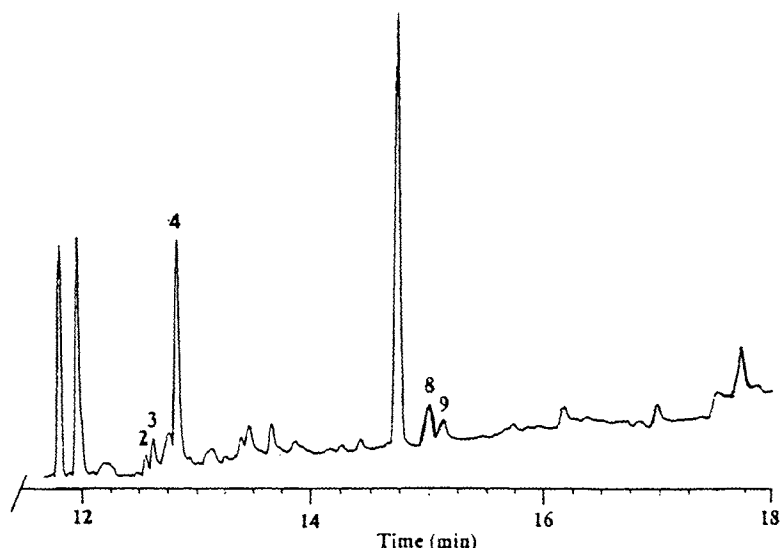


Fig. 4.28. Gas chromatogram of a ground water well sample extracted by SPME (85 μm polyacrylate fibre). From Ref. [231] with permission. Analytes: (2) simazine; (3) atrazine; (4) propazine; (8) simetryn; (9) ametryn. The concentrations of these identified triazines were 0.05–0.3 $\mu\text{g/l}$.

preferably by GC-MS. But this example has been selected to show that in surface water, although some interferences are showing up, the determination of organochlorine pesticides can still be performed at low levels. Humic and fulvic substances do not seem to be extracted by the polydimethylsiloxane fibre, or only to a small extent. This results from the basis of the SPME, which is not an exhaustive method but an equilibrium method. In exhaustive methods, such as LLE or most of SPE, the first aim is to obtain quantitative transfer of target analytes into the extracting phase in order to have 100% recoveries, and a consequence is that selectivity is often lost because many matrix components are co-extracted. Equilibrium methods are more selective because they take full advantage of differences in extracting-phase-matrix distribution constants to separate target analytes from interferences [228].

4.2.4.4. Conclusion

SPME is certainly a promising new and solvent-free method for the handling of aqueous samples prior to GC analysis. Its important features are its simplicity, low cost, rapidity and the sensitivity of the combination of SPME-GC with appropriate detection modes. It requires small amounts of samples, which may be attractive for applications where the sample volume is limited. The devices based on polymer technology are easily adapted for simple and direct introduction of concentrated samples into the analytical instruments, and automation is easy and available. Line-

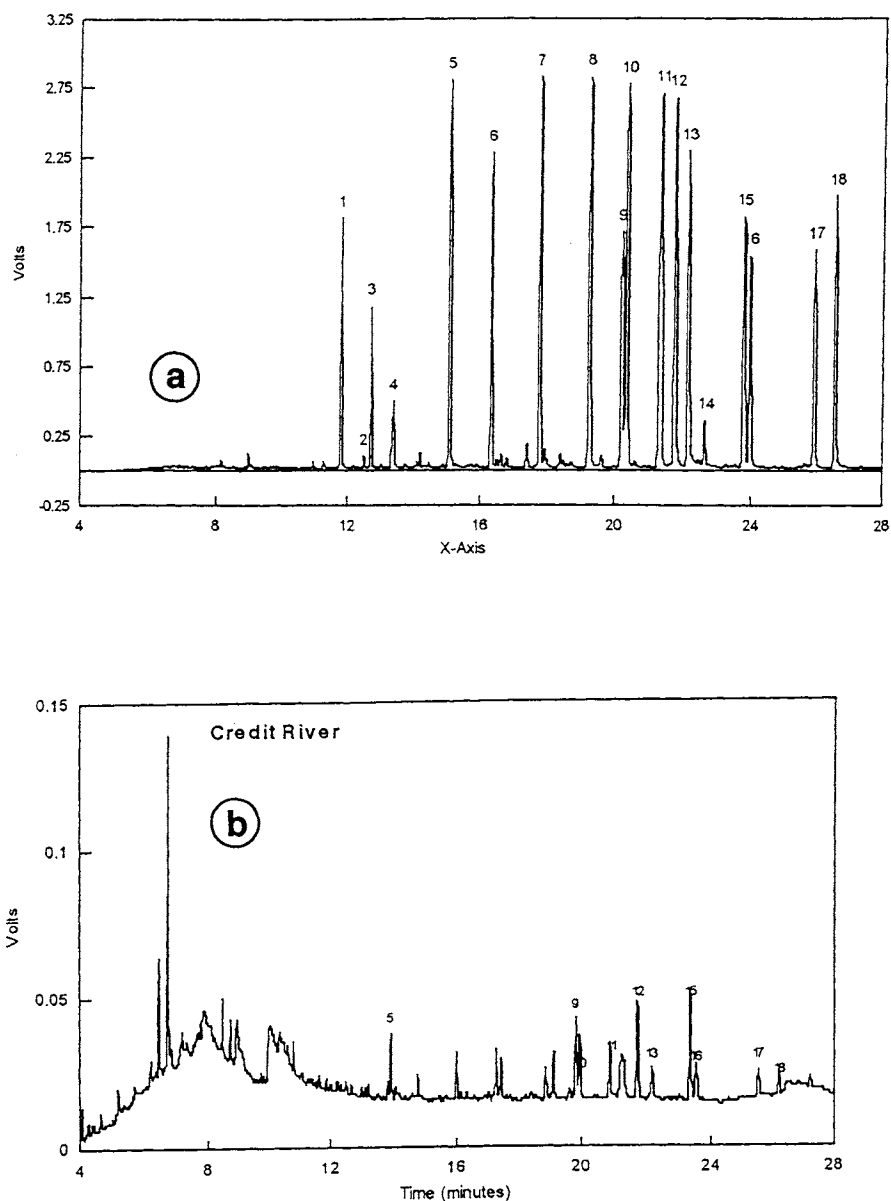


Fig. 4.29. (a) Analysis of a 1 ppb ($\mu\text{g/l}$) aqueous standard using SPME (100 μm polydimethylsiloxane fibre and GC-ECD and (b) analysis of a river sample under the same experimental conditions. from Ref. 230. Analytes: (1) α -HCH; (2) β -HCH; (3) γ -HCH (lindane); (4) δ -HCH; (5) heptachlor; (6) aldrin; (7) heptachlor epoxide; (8) endosulfan I; (9) dieldrin; (10) *p,p'*-DDE; (11) endrin; (12) endosulfan II; (13) *p,p'*-DDD; (14) endrin aldehyde; (15) endosulfate; (16) *p,p'*-DDT; (17) endrin ketone; (18) methoxy-chlor.

arity is obtained over a wide range of concentration, and the detection limits are in agreement with regulation levels. As it is based on an equilibrium, recoveries are not 100%, but the examples presented above have shown that precision and reproducibility are similar to other conventional extraction methods. Moreover, one advantage over exhaustive extraction method is a reduction in the co-extraction of humic substances.

The current limitations are the fact that there are limited types of fibre, especially for the extraction of the more polar pesticides. More specificity and selectivity are expected with the constant progress in polymer technology. Fused silica fibres coated with graphitized carbon black have been tested [236]. A new fibre coated with Carbowax/divinylbenzene (65 μm) has just become available and has been shown to extract polar compounds such as alcohols from blood matrices [237]. SPME has been shown to be a method for estimating the octanol–water partition coefficients [238]. Further developments are certainly to be expected in the near future with this new approach.

4.2.5. Supercritical phase extraction (SFE)

In the last 5 years, supercritical fluid extraction (SFE) has proved to be an appropriate replacement for the Soxhlet extraction of solid samples. The latter requires several hours to perform and uses large volumes of pesticide-grade organic solvents which give dilute extracts that have to be concentrated and often need thorough clean-up before analysis. Many recent papers discuss SFE, method development with spiked samples, and environmental applications to the trace analysis of organic pollutants in real samples. For reviews, see refs. [239–243]. SFE has gained acceptance for the handling of complicated real matrices because it gives recoveries equal to, and sometimes higher than, traditional extraction procedures in certification procedures on environmental matrices. It achieves the aim of the US EPA to considerably reduce the use of organic solvents. Another consequence was the rapid development of SFE instrumentation.

Most applications deal with solid matrices (soil, sediment, food, etc.) and not with aqueous matrices. The most important characteristics of SFE are the high recovery rates which can be obtained in a relatively short time, typically ca. 30 min, and the selectivity that can be introduced in different manners, as can be seen below. This selectivity is the basis of its application to environmental water. Although direct extractions from aqueous matrices have been described which add an adsorbent in the extraction cell [244–248], SFE of aqueous samples is more often described as a combination of a first SPE of pesticides on non-selective solid sorbents (cartridges or disks) and a subsequent fast SFE of the compounds adsorbed on them. The tandem SPE-SFE has been shown to be a more selective extraction procedure than SPE alone [244,249–255]. In addition, particle-laden samples can be extracted in one step and the particles need not be filtered and extracted separately. This was shown to be

very efficient for replacing the EPA method 608 for the determination of chlorinated pesticides in water and waste water, where approximately 1 l of water was extracted with at least 200 ml of dichloromethane in each analysis. It was calculated that this method alone, one of the most widely used of the 600 series, was responsible for the release of at least 10^5 kg/year of dichloromethane into the environment [251].

To set up an efficient SFE requires several steps. The tandem SPE-SFE is easier to perform than SFE alone with native solid matrices, since SPE disks or cartridges with adsorbed analytes are more close to spiked solid samples than to native solid samples. In general, good recoveries are obtained with spiked solid samples because the analytes are situated on the surface of sample matrices and have little time to migrate to strong binding sites. In contrast, in real solid samples the native pollutants have often been in contact with the matrix for years and can be associated with stronger binding sites than in the spiked samples [243].

In this section, we explain the importance of some basic parameters for correct performance of a SFE sequence and present some applications using tandem SPE-SFE.

4.2.5.1. Description and optimization of extraction conditions

SFE can be performed in various modes. In the static mode, the sample is in contact with a static supercritical fluid in a closed container, whereas in the dynamic mode the fluid is percolated through the sample. In most instances, extractions combine these two modes, since a short period of time is allowed for static equilibration before enabling the sample to be dynamically extracted [239]. Once extracted from the matrix, the solutes of interest are usually trapped by a sorbent before analysis.

The SFE sequence can be divided into three steps, i.e., initial partitioning of the analytes from the matrix sites into the supercritical fluid, elution of the analytes from the extraction cell, and collection of the analytes in the SFE trapping system [256]. The proper development of quantitative SFE is greatly helped by an understanding of these three steps, on which we comment briefly. Selectivity can be introduced by several means: (i) control of the pressure and temperature during the extraction; (ii) addition of a polar modifier; and (iii) selection of the trapping sorbent and of washing conditions before desorption.

4.2.5.1.1. Fluids in SFE. Supercritical fluids possess unique physico-chemical properties which make them appropriate as extraction solvents. Their viscosity is 5–20 times smaller than that of liquids, so the diffusion coefficients of solutes are greater which provides a means for more rapid and efficient penetration of solid matrices. They also have densities 100–1000 times greater than the usual gases, which gives them solvating powers close to those of liquids. In addition, their density is related to the pressure, and to a lesser extent to temperature, so that their solvating ability can be easily modified by changing the extraction pressure and/or temperature [239].

By far the most widely used extraction fluid is supercritical CO₂ (more than 90% of analytical SFE), because it is chemically inert, inexpensive, non-toxic, non-flammable, non-explosive, and has an easily accessible critical point at 31.1°C and 72.8 bar. It is easily removed from the extract and creates no environmental problem when used for analytical purposes. Extraction can thus be performed under mild thermal conditions. In the supercritical state, CO₂ has a polarity comparable to liquid pentane and is therefore best suited to the extraction of hydrophobic pesticides; its main drawback is its lack of polarity for the extraction of polar pesticides. Fluid N₂O has been shown to be more appropriate for polar compounds because of its permanent dipole moment, but has also been shown to cause violent explosions when used with samples having high organic content [257]. Freons and SF₆ have also been used for the extraction of apolar analytes such as PAHs or PCBs from river sediments, with higher recoveries than pure CO₂ and CO₂-modified with methanol [258]. Supercritical H₂O is, of course, an interesting fluid and has often been used for the destruction of hazardous organics [243]. Its main drawbacks are the high temperature and pressure needed ($T > 374^{\circ}\text{C}$ and $P > 221$ bar) and the resulting corrosive nature of water in these conditions. Hawthorne et al. have shown that subcritical H₂O (250°C and 350 bar) was able to extract PAHs quantitatively from certified soil and air particulate samples in 15 min, as well as more polar compounds such as chlorinated phenols at lower temperatures [259,260]. Although this fluid will certainly be used more in environmental analysis in the near future, its application to pesticide analysis will be restricted to the thermally stable materials, and we can predict that CO₂ will remain the fluid of choice for pesticide analysis.

4.2.5.1.2. Important parameters

Temperature and pressure. The two basic parameters are the extraction recovery (usually expressed in mass percentage) and the extraction rate (extraction recovery per unit of time, at a given velocity of the supercritical fluid through the cell). The realization of a SFE requires the optimization of several parameters, mainly the pressure and temperature, the possible addition of an organic modifier to the fluid, and the flow rate [239].

Pressure and temperature are the two main parameters that influence the extraction recovery. Together, they define the density of the supercritical fluid, as shown by the well-known pressure-density diagram at different temperatures [239]. The maximum extraction fluid density is obtained with high pressures at temperatures close to the critical temperature of the fluid. An increase in pressure at a given temperature results in an increase in the density of the fluid, which means a better solubility of the solutes, as illustrated in Fig. 4.30 which shows the percentage recovery of atrazine from soil by extraction with CO₂ at different pressures after 15 min at 80°C and constant flow rate, compared with the solubility of atrazine in CO₂ at the same temperature [261]. However, one is not always recommended to increase the pressure too much for complex matrices because more interferences will also be co-

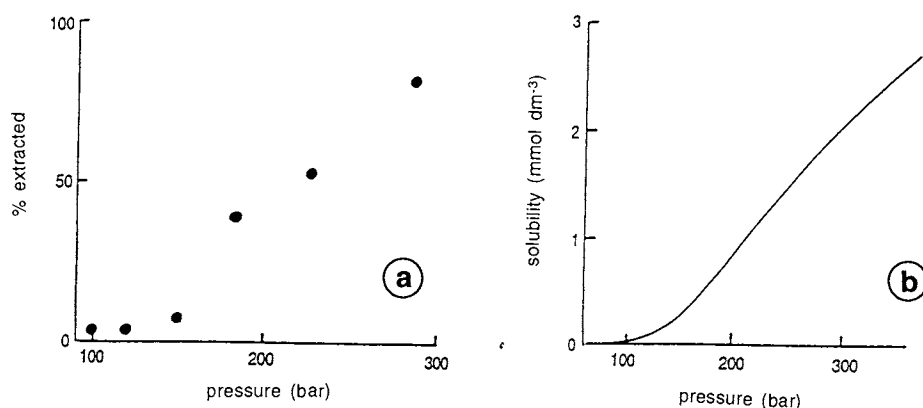


Fig. 4.30. (a) Percentage recovery of atrazine from soil by extraction with CO₂ at different pressures after 15 min at 80°C and constant flow rate, (b) compared with solubility of atrazine in CO₂ at the same temperature. From Ref. [261] with permission.

extracted, resulting in a loss of selectivity. At constant pressure, the density of the fluid reduces with an increase in temperature so that, for a non-volatile analyte, a higher temperature should result in a lower recovery owing to a reduction in solubility. However, it is important to point out that the solubility of a substance in a supercritical fluid is affected by both the volatility of the substance and the solvating effect of the fluid, which is related to its density. Therefore, an increase in temperature of the fluid can increase the solubility of compounds with significant vapour pressures. For example, the extraction recovery of diuron from soil with methanol-modified CO₂ was enhanced from 75 to 99% when the temperature increased from 80 to 120°C [239,262]. Dramatic increases in SFE recoveries have been observed when raising the temperature alone [263,264]. For example, at 50°C, raising the extraction pressure of pure CO₂ from 150 to 659 bar did not increase the recoveries of PAHs and PCBs in certified reference materials. The compounds were efficiently extracted only if the temperature was raised to 200°C and although PCBs were extracted from a sediment at 200°C with any pressure between 150 and 659 bar, the recoveries of PAHs still increase with the pressure and the temperature [263]. The recoveries of various analytes – PAHs, N- and S-heterocyclics, chlorinated phenols and pesticides from a variety of soil and soot samples – have been measured using pure CO₂ (30 min) and compared to recoveries obtained with 18 h Soxhlet extractions [263]. For all the samples studied, increasing the extraction temperature from 50 to 200°C yielded significant increases in recoveries despite the reduction in CO₂ density from 0.93 to 0.50 g/ml. Recoveries of three pesticides (atrazine, prometon and metolachlor) were improved from 30 to 40% at 200°C, in comparison to those obtained at 50°C, and were between 110 and 120% (versus Soxhlet experiments). Further in-

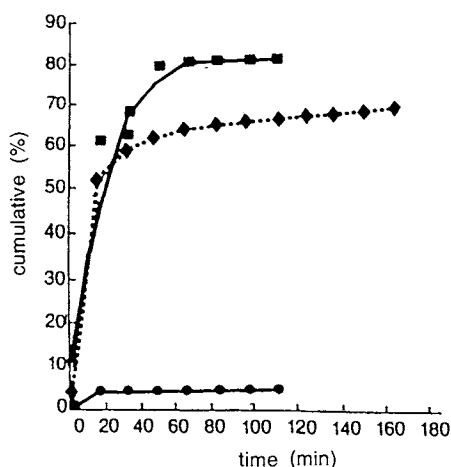


Fig. 4.31. Effect of the modifier on the extraction of diuron from a soil at constant pressure (340 bar) and temperature (100°C). Flow rate, 5 ml/min. (●) 1.25% methanol, (■) 10% methanol, (◆) 10% acetonitrile. From Ref. [265] with permission.

creases in the SFE temperature to 350°C did not yield increased recoveries but did show evidence of causing thermal degradation.

Addition of modifiers. Since CO₂ is relatively non-polar, a few percent of a polar modifier is often added to enhance the solubility of the more polar compounds or to increase the ability of the fluid to better displace analytes from matrix sites. The modifier can be added directly to the sample in the cell, but then a static mode is needed (if not, it would lead to a concentration gradient within the matrix). This method is useful for rapidly evaluation of the effect of various modifiers. Premixed fluids, prepared with the addition of the modifier to the liquid CO₂ can also be used, but the most accurate and reproducible method is to use a separate modifier pump, provided that the system compensates for the compressibility, and therefore density, of the CO₂ [243]. Various modifiers have been tried over recent years, but methanol remains the most popular. The nature of the modifier depends on the nature of the analytes to be extracted. Predictions are difficult, and there is no clear understanding of the specific mechanism that operates, apart from the enhancement in solubility. A reasonable starting point for the selection of the modifier is that in its liquid state it should be a good solvent for the target analyte. As can be seen in Fig. 4.31, the extraction of diuron is greatly enhanced with methanol instead of acetonitrile as a modifier, probably because hydrogen bonding can occur between diuron and methanol [239,265]. The effect of the amount of modifier on recoveries is important. Langefeld et al. have recently studied the effect of eight different modifiers (methanol, methylene chloride, toluene, methanol–toluene, hexane, aniline, diethylamine, and acetic acid) at concentrations of 1 and 10%, and the results indicated that the

identity of the modifier was more important than its concentration [266]. The effect of polar modifiers is not really well known; they can affect the matrix by covering the active sites and preventing re-adsorption of analytes onto the matrix, and also by reducing the interaction energy between the matrix and the solute.

Water also acts as a modifier. Many environmental samples contain water which may either help or hinder the extraction process, but the most important effect is to cause restrictor plugging; the most obvious way to avoid this is to dry the sample. However, both oven and room temperature drying have been shown to lead to substantial losses of analytes [243]. A drying agent can also be mixed into the sample, but this procedure can also lead to substantial losses of volatile and semi-volatile analytes since mixing a wet sample with a drying agent can cause heating. Although the role of water is still unclear, it appears that a small amount of water (e.g., 1–2%) is advantageous but that greater amounts may either increase or reduce the extraction efficiencies [243].

The addition of a polar modifier is required for moderately polar and polar pesticides. Papilloud and Haerdi have studied the SFE of atrazine and polar metabolites, such as hydroxyatrazine and de-ethyl-de-isopropylhydroxyatrazine by spiking a C₁₈ silica sorbent. The optimal condition for 90–95% recoveries of atrazine and hydroxyatrazine were a pressure of 250 bar at 50°C, the addition of 10% (v/v) methanol, and a 45-min extraction time at a flow rate of 0.5 ml/min. Under these conditions, the recovery of the very polar de-ethyl-de-isopropylhydroxyatrazine was only 20% [267,268]. When using 10% methanol that contained 2% of water (v/v), the recovery increased to 52%. SFE has also been shown to provide an alternative to polar organic solvents or water in the quantitative removal of atrazine and the two dealkylated metabolites, cyanazine and metolachlor from agricultural soils [269]. The addition of both water and methanol to the air-dried soil prior to commencement of the CO₂ flow strongly enhanced the recoveries but did not promote the conversion of the chlorotriazine analytes to any of their corresponding hydroxy- or methoxy-analogues under the SFE conditions tested.

Fluid velocity. The flow velocity of the supercritical fluid through the cell has a strong influence on the extraction efficiency, depending on whether this is limited by solubility considerations or by the kinetics of the desorption process [270]. A slow flow will ensure a deeper penetration of the fluid into the matrix. The speed of flow is usually expressed by the linear velocity, which depends on the flow rate and the cell geometry. Small cells are mainly used, and are more reliable, so the samples are typically in the order of 5–10 g. A minimum of 4–5 cell volumes is needed at least to sweep the cell void-volume sufficiently. If the extraction rates are limited by solubility considerations, the increase of the flow rate should lead to substantially higher recoveries, since in a given time a larger amount of supercritical fluid will be applied. If the kinetics of the desorption of the solutes into the supercritical fluid limit the extraction rate, an increase in the flow rate will have no significant effect on the recoveries, since the sample is exposed to the extraction fluid for the same time.

In practice, a high flow rate may result in a reduction in the recovery by increasing the loss during decompression of the fluid. Typical values are around 1 ml/min of compressed fluid for an extraction cell of i.d. ca. 1 cm, which corresponds to ca. 500 ml/min of gas after decompression [239].

Matrix effect. Factors such as the particle-size, shape, surface area, and porosity of the solid sample, and the nature of the matrix, will affect the analytical results and are of prime importance. However, in the SFE of a SPE sorbent which has been loaded with aqueous samples, the effect of the matrix is expected to be small in comparison with that for native solid samples.

4.2.5.1.3. Efficiency of the analyte trapping system. Once the analytes of interest are in the supercritical fluid they have to be isolated for further analysis. Generally, this occurs by decompression of the fluid through a restrictor, which needs to be heated at 5°C to prevent the restrictor plugging with ice formation. In off-line techniques, a liquid trap or a solid surface are commonly used for collecting the extract.

With a liquid trap, the restrictor is simply placed in a vial containing a suitable solvent. Obviously, the selection of the collection solvent is important. Methylene chloride, acetone or chloroform have been shown to be more appropriate than methanol and hexane for a varied set of organic pollutants [271]. The collection of relatively volatile substances can be difficult and losses of polycyclic aromatic hydrocarbons of up to 30% have been observed with different collection devices [272]. The use of a Dewar condenser placed on top of the SFE off-line collection vials was shown to reduce analyte losses significantly [273]. A solid-liquid trap was shown to minimize these trapping problems. Solid-phase trapping is usually carried out by depressurizing CO₂ and the analytes prior to their collection onto a sorbent such as silica, Florisil or bonded silicas. After being cryogenically and chemically trapped, the solutes are eluted from the sorbent with a small volume of solvent. This technique is less straightforward than the liquid trapping, since its optimization requires the selection of the sorbent, trapping temperature, and elution solvent. Several new commercial instruments utilize this approach, so it will gain more attention. The liquid and solid trapping systems have been compared for the analysis of hydrocarbons [274]. It was shown that the average recoveries were increased by ca. 20% by using the solid-liquid trap, without loss of the less volatile analytes or dependence on the flow rate of the extraction fluid. Silicagel and C₁₈ bonded silicas showed no difference in adsorbing the analytes. Separate analysis of the organic solvent and the solid traps after extraction with pure supercritical CO₂ showed no PAHs in the organic solvent whereas when toluene was added as modifier, the analytes were not found in the sorbent but in the organic solvent. Moreover, this approach has the advantage over liquid trapping of allowing fractionation or clean-up during the desorption from the trapping solid [274].

On-line collection can also be performed by subsequent analysis with GC, LC, or SFC, and is discussed in the next chapter with other on-line techniques.

4.2.5.2. Application to aqueous matrices

4.2.5.2.1. Direct extraction. Direct SFE is possible but is associated with several problems. First, the nature of the sample necessitates the use of special extraction cells. Then, the main problem is the relatively high solubility of water in supercritical CO₂, approximately 0.3%, which may cause restrictor plugging by ice. The first studies used a “close loop stripping” method, where the fluid was recirculated by a pump back into the water sample [245]. Another method included a phase separator to remove the water from the CO₂ before analysis by SFC [246].

Barnabas et al. [244] have recently optimized the SFE conditions for the removal of organochlorine pesticides (OCPs) from water using a modified “headspace” extraction cell and compared the results with the tandem SPE-SFE. Despite the good solubility of OCPs in pure CO₂, the recoveries decreased when they were directly extracted from water. Increasing the flow rate between 0.7 and 1.5 ml/min, with a typical extraction time of 45 min, had little effect, and the extraction was not affected by the addition of salt to the matrix. Maximum recoveries of lindane, aldrin and dieldrin were around 40–70% with 100 min extraction and were about 20% lower than from an extraction involving prior trapping of these analytes onto a C₁₈ extraction disk using the combined SPE-SFE method. These lower recoveries may be indicative of a poor diffusion of the supercritical CO₂ through the aqueous matrix.

Direct extraction of phenoxyacetic and phenoxybenzoic acids from water using supercritical CO₂ has been described [247]. The effect of different pressures, temperatures and modifiers has been investigated, showing that the addition of a mixture of acetonitrile and water was more efficient for the extraction of phenoxybenzoic acids whereas supercritical CO₂ modified with methanol and water yielded more efficient extraction of phenoxyacetic acids. Another approach described the simultaneous extraction and methylation of chlorophenoxyacetic acids from aqueous solution using supercritical CO₂ containing methyl iodide and tetrahexylammonium hydrogen sulfate [248]. Conversion to methyl ester derivatives was achieved in 30 min with good recoveries from 1 ppm solutions.

4.2.5.2.2. SPE-SFE. This is certainly the most appropriate method for applying the SFE selectivity to aqueous samples. The preliminary extraction of analytes can be performed directly in the SFE cell which has been previously filled with an appropriate sorbents or separately. However, in most examples, Empore C₁₈ disks have been used to trap the analytes initially, prior to elution with pure or modified supercritical CO₂. The first step is similar to that previously described in the SPE section. After percolation of the sample, the disk is first air-dried for several minutes, and additional drying in an oven at 40–50°C is often performed for at least 30 min. Then, the disk is rolled and placed in a 10 ml or smaller extraction cell. The integrity of the SPE disk under supercritical fluid extraction was investigated and the collected extract was analyzed by GC-EC-MS leading to chromatograms free of any contamination [251].

TABLE 4.18

PERCENT RECOVERIES OF ORGANOCHLORINE PESTICIDES AND UREA AND TRIAZINE HERBICIDES USING CO₂ ALONE AND CO₂ MODIFIED WITH 10% METHANOL (ADAPTED FROM REF. [253] WITH PERMISSION)

Compounds	Recoveries using pure CO ₂ ± SD)	Recoveries using modified CO ₂ ± SD
<i>Organochlorines</i>		
Heptachlor	92 ± 2	
Isodrin	102 ± 8.3	
Dieldrin	85 ± 2.4	
<i>Herbicides</i>		
Simazine	1.6 ± 1.2	100 ± 13
Propazine	2.7 ± 4.5	89 ± 7
Trietazine	3.5 ± 3.4	87 ± 8
Chlortoluron	4.4 ± 3.1	86 ± 8
Isoproturon	3.9 ± 2.1	90 ± 5
Diuron	4.3 ± 2.6	88 ± 7

Good recoveries have been obtained for aldrin and dieldrin with a static extraction of 30 min followed by a dynamic extraction with pure CO₂ at 300 bar and 50°C [244]. A lower recovery was obtained for lindane with the same conditions, but the authors were unable to determine whether this was a results of poor extraction on the SPE disk or of the SFE of the disks. Another publication describes the determination of chlorinated pesticides and PCBs in water and wastewater with recoveries of at least 80% -which are around 10% higher than those obtained with SPE alone [251]. The advantage was that the particles filtered from the water could be extracted with the SPE disk.

The quantitative SFE of sulfonylurea herbicides (sulfuron methyl and chlorsulfuron) from aqueous matrices via SPE with C₁₈ disks has been described [249]. A Celite™ matrix was used first to determine optimum conditions for extraction and to see whether the analytes were, indeed, quantitatively extractable by SFE without the possibility of matrix interferences. Both herbicides were quantitatively extracted with supercritical 2% methanol-modified CO₂ from the Celite matrix and water, via solid-phase extraction. The optimized SFE conditions were 50°C, 350 bar, a flow rate of 2ml/min and a 2 min static extraction followed by a 24 min dynamic extraction. Stainless-steel beads were used as the trapping material and elution was performed with acetonitrile. Comparisons were made with traditional organic solvent-elution of the SPE disk with 1-l samples spiked with 50 µg/l of both sulfuron methyl and chlor-sulfuron, but no evidence is shown for a higher selectivity using the SPE-SFE procedure.

Fractionation. Selectivity can be introduced when using SPE and a fractionation of organochlorine pesticides from phenylurea and triazine herbicides was obtained

by making two subsequent extractions of the SPE disk under different conditions [253]. A 200 ml aliquot of a spiked water sample was extracted on a C₁₈ Empore disk which was dried at 45°C for 20 min. The disk was then placed in a 10 ml extraction cell at 50°C and extracted at 250 bar with CO₂ at a flow rate of 2 ml/min. The extract containing the organochlorine pesticides was collected in hexane. The disk was then re-extracted under identical conditions but with the addition of 10% methanol. The herbicide fraction was collected in the HPLC mobile phase (55% methanol–water mixture, v/v). Table 4.18 shows the selectivity that was obtained within the two groups since the first extraction removes only the organochlorine pesticides whereas the second quantitatively removes the herbicides.

A fractionation has also been obtained by adjusting the pressure of the supercritical CO₂; the method was applied to the determination of pesticides in waste water [254].

Selectivity from the matrix. Few studies have shown the selectivity that can be obtained in using SFE for aqueous samples. Alzaga et al. examined the stability of freeze-dried samples spiked with eight pesticides for evaluating their suitability as candidate reference materials for their determination in water samples [275]. The SFE procedure was performed by introducing the freeze-dried residue within the SFE cell. The freeze-dried extract was reconstituted and liquid–liquid extraction was performed as a comparison. SFE recoveries of at least 90% were obtained at 50°C, 200 bar and 30 ml of CO₂; no modifier was required for these lyophilized extracts of spiked surface water samples. The characteristic LC-DAD chromatograms are shown in Fig. 4.32 where the higher selectivity of the SFE is apparent. In LLE, simazine co-elutes with an interfering substance and glycine which was added as a stabilizer was extracted by LLE but not with SFE. Moreover, analysis by GC-NPD was possible with the SFE extract whereas a further clean-up was required for the LLE extract.

Selectivity was shown with the SFE of several organophosphorus pesticides in a spiked orange juice sample [252]. Initial attempts to extract the aqueous sample, as such, failed and the sample was added to a solid (Chromosorb W) packed in the extraction thimble. The SFE was performed at 50°C with 0.5 ml/min of pure CO₂ at a density of 0.75 g/ml during 5 min of static- and 30 min of dynamic extraction. The extracted analytes were collected on a C₁₈ silica trap held at 20°C and recovered after rinsing the trap with 1 ml of chloroform. Further analysis was performed by GC-MS. Under these moderate extraction conditions, a relatively clean chromatogram was obtained, with palmitic and oleic acids as the main peaks from the matrix. The selectivity of the SFE was fully exploited since both the silica sorbent in the thimble, and the C₁₈ silica sorbent as the collection trap, helped to retain unwanted matrix solutes, polar compounds such as lower free fatty acids, and high-mass molecular solutes such as triglycerides. The method was automated by positioning the SF extractor such that the GC automatic sampler arm of the injector could provide and withdraw vials from the SFE instrument. The total analysis time was approximately 1 h, and

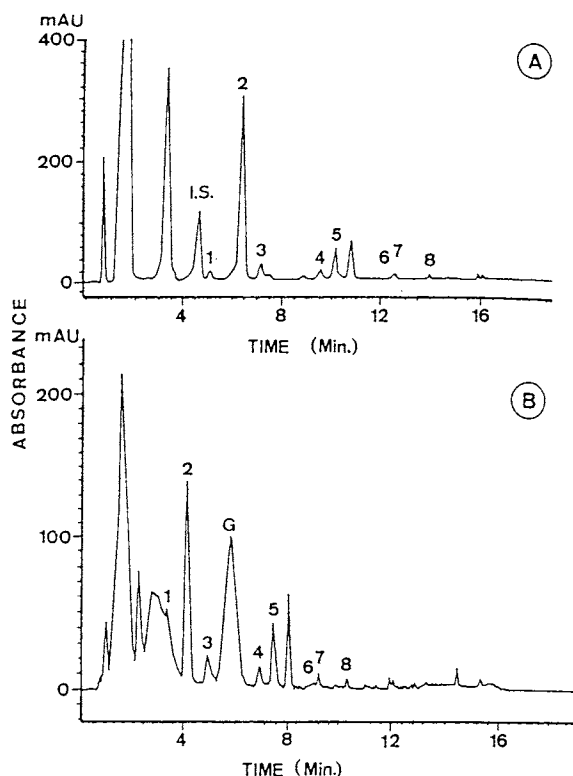


Fig. 4.32. LC-DAD (220 nm) chromatograms of extracts isolated by (A) SFE (CO₂, 50°C, 30 MPa, 15 ml CO₂) and (B) LLE (internal standard IS, cyanazine). Compounds: (1) simazine; (2) carbaryl; (3) atrazine; (4) propanil; (5) linuron; (6) fenitrothion; (7) fenamiphos; (8) parathion-ethyl; G; glycine. From Ref. [275] with permission.

was much more rapid than the conventional liquid–liquid extraction followed by a clean-up over silica-Florasil, or by size-exclusion chromatography.

4.2.5.3. Conclusion

Obviously, SFE of aqueous samples is possible and relatively easy to perform when combined with a SPE step, although setting up an efficient SFE requires a good knowledge of the effect of the basic parameters. The selectivity of the SPE-SFE process compared to the SPE alone is certainly the main interest in the technique. It has proved to be competitive in terms of both accuracy and precision for the certification of reference materials [276]. Another advantage is the possibility of performing the SPE step directly in the field, using conventional sorbents, and carrying out the SFE step in the laboratory, as a consequence of the good preservation of many analytes when they are adsorbed on C₁₈ or polymeric materials.

This approach has been applied with success to the control of rotenone in the Ohio river [250].

Future applications to aqueous samples will depend greatly on the implementation of SPE for the sample pretreatment of soils, sediments, and other solid matrices such as plant tissues or foodstuffs. Other techniques are now emerging, such as accelerated solvent extraction procedures under pressure and high temperature, and microwave-assisted extraction procedures which reduce the use of organic solvents [277]. However, SFE has the advantage of being a solvent-free technique. We can expect the subcritical water to be used more in the future, even if CO₂ is now considered as the “greener solvent” [278]. EPA has recently accepted the use of supercritical fluid CO₂ in extracting the total petroleum hydrocarbon and polycyclic hydrocarbons under SW846 (Method 3560 and 3561). Further developments are also expected in combination with those performed for the selective clean-up and/or detection. For polar and thermolabile pesticides, the combination of extraction by supercritical fluids with enzyme immunoassays has been shown to allow faster extraction and analysis, without further clean-up [279].

4.3. CLEAN-UP PROCEDURES

As seen previously in this chapter, in most cases clean-up is not necessary for ground and drinking water. For more complex matrices such as surface-, runoff- or soil water samples, selective extraction procedures offers an elegant solution since, in one step, pesticides are extracted and concentrated without a requirement for clean-up. However, although this is an active area of research, the methods most currently used are non-selective LLE or SPE which yield an extract that often contains too many interfering compounds for easy analysis by chromatography at a trace-level without additional clean-up.

The need for clean-up depends on many factors such as the type of water, the concentration levels, the separation performed, and the degree of selectivity in the detection mode. Clean-up can be introduced into the SPE sequence as described in Fig. 4.4 or can be applied to the dry water extract using adsorption or size-exclusion chromatography. These last two methods have been extensively described for more complex matrices such as food or soil, and no difference exists between the clean-up process applied to dry extracts of soils, water or food.

4.3.1. Clean-up included in the SPE sequence with non-polar sorbents

With hydrophobic sorbents, the clean-up step can be included in the SPE sequence just before the analyte desorption (see Fig. 4.4). It is performed by flushing the SPE cartridge with a small volume of water modified with an organic solvent so that many matrix components are eluted, but not the analytes of interest. In fact, this flushing can only remove interferences which are more polar than the analytes of in-

terest, so the method can only be applied for the determination of hydrophobic pesticides. It is difficult to apply it for multiresidue analysis. In such cases, if some polar analytes are also to be determined, it is very important to verify that no loss occurs with this flushing solvent.

Another means for removing some polar interferences is to add some organic solvent to the aqueous sample before percolation through the sorbent. Again, this procedure can only be applied for the determination of non-polar pesticides. The breakthrough volume will not be estimated from $\log k'_w$ values but from $\log k'$ values obtained with that concentration of organic solvent. Figure 4.9, which shows the linear and/or quadratic relationship between $\log k'$ and the percentage of methanol in the mobile phase, indicates that the addition of 5% of methanol can considerably reduce the breakthrough volume.

The two approaches mentioned above were applied by van der Hoff et al. [116] for the simultaneous extraction and clean-up of pyrethroids in the analysis of surface water, integrated into an on-line procedure. Some 30% of methanol was added to the sample prior to SPE and the cartridge was washed with an additional 1 ml of 30% methanol in water before on-line desorption by toluene and GC-ECD analysis. This clean-up was shown to be efficient and led to detection limits in surface water below the $0.1 \mu\text{g/l}$ level.

Fractionation can occur by coupling different sorbents when the clean-up cannot be performed after sample application and before analyte elution. Di Corcia and Samperi [162] have shown that selective SPE can be performed by coupling two sorbents. The first cartridge packed with the non-specific graphitized carbon black sorbent, traps the analyte of interest and many other compounds, but only basic analytes are transferred and re-concentrated into a second cartridge packed with a more specific sorbent such as a cation exchanger. They could then determine chlorotriazines at the ppt level. By percolating 2 l of drinking water through a cartridge packed with 250 mg of graphitized carbon black, and then connecting this cartridge to a second one packed with a cation-exchanger and flushing the two columns with a mixture of dichloromethane and methanol, these authors have also determined 14 phenylurea herbicides in drinking water with detection limits at the ng/l level. Phenylureas are eluted while all basic interferences such as chlorotriazines, and anilines are trapped by the ion-exchanger [162].

4.3.2. Clean-up using polar sorbents (adsorption chromatography)

4.3.2.1. Basic principles

A widely used clean-up method is based on the fractionation of the extracts using polar LC sorbents such as silica, alumina, Florisil (synthetic magnesium silicate), or silica chemically modified with amino groups. The basic principle which governs this process is adsorption chromatography and is described in Fig. 4.33. The extract

is dissolved in a non-polar solvent, typically hexane, and percolated through the polar sorbent. Step-elution with solvents of increasing polarity allows a separation into fractions on the basis of polarity differences. Such a fractionation was described by Valls et al. [280], for the determination of ionic and non-ionic contaminants in urban waste and coastal waters. The fraction F1 was eluted by hexane and contained aromatic hydrocarbons. By adding increasing percentages of methylene chloride, methanol and diethyl ether in the eluting mixture, the authors obtained seven fractions containing linear alkylbenzenes and polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), and waxes, fatty acid methyl esters, alkyl- and aryl phosphates, and ketones, sterols, and nonylphenol polyethoxylates in the last eluted fraction. This analytical procedure contains many steps and is very time-consuming. The only advantage is its broad screening for the identification of unknown compounds.

4.3.2.2. Clean-up with SPE cartridges or home-made SPE columns

4.3.2.2.1. Sorbents. Clean-up of extracts is based on a fractionation scheme similar to that described above. Dry extracts are dissolved in hexane and compounds are retained on the polar sorbent. Then, one has to find the appropriate solvent mixture which only elutes the analytes of interest. The most common sorbent is Florisil which has been successfully used for clean-up of water and/or soil extracts for the

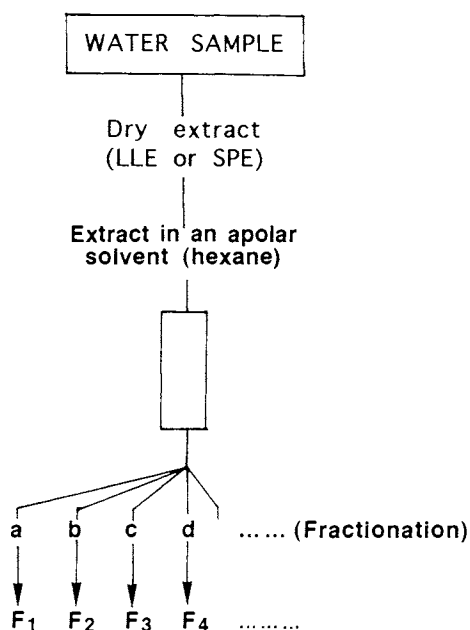


Fig. 4.33. Typical scheme for the fractionation or clean-up of an extract. After injection of the extract, fractionation occurs by eluting the column with eluents a–d, etc., of increasing polarity.

determination of various pesticides [281–284]. Silica gel has also been used, mainly in the determination of rather non-polar pesticides such as organochlorine, organophosphorus or pyrethroid pesticides [281,285,286]. Alumina in its different forms (neutral, acidic or basic) has been used less extensively, and only for the determination of organochlorine pesticides [287].

A relevant example is the clean-up of water samples for the determination of organochlorine and pyrethroid insecticides using silica cartridges [23]. The extract was obtained from 15 ml of surface water by LLE with hexane and was further evaporated down to 1 ml. Clean-up was made with a 100-mg silica cartridge and the whole sequence -i.e., conditioning with 2 ml of hexane-isopropyl alcohol (80:20, v/v) and 6 ml of hexane, application of 500 μ l of the extract, washing with 1 ml of hexane and desorption with 1 ml of hexane-toluene (70:30, v/v)- was automated by the ASPEC (Automatic Sample Preparation with Extraction Columns) from Gilson, which was coupled on-line to capillary GC-ECD by means of a loop-interface equipped with a solvent vapour exit. The complete analytical procedure was greatly facilitated by automation with a considerable reduction in the sample volume required, and the determination of synthetic pyrethroids at ppt levels in surface water. Figure 4.34 shows the difference in the chromatograms obtained with and without clean-up.

The clean-up step can also be based on normal phase chromatography using cartridges packed with silica modified with polar aminopropyl groups. This was applied with success to the determination of *N*-methylcarbamates in soils, vegetables and fruits [288,289].

4.3.2.2.2. Removal of humic and fulvic acids in surface water extracts. For contaminated surface water samples, the clean-up step is required to remove humic and fulvic acids. Figure 4.15 shows that when a C_{18} cartridge was used for the extraction and concentration of acidic herbicides, the co-extraction of humic and fulvic acids did not permit detection limits below the 0.5 μ g/l level in surface water although the same procedure gave detection limits in the low to 0.1 μ g/l level in drinking water samples. A clean-up procedure was described which uses a Florisil cartridge [114]. A practical problem was that after percolation of 500 ml of surface water sample through a 500 mg- cartridge, it was impossible to dissolve the dry extract in pure hexane, even by increasing the volume to 20 ml. A moderately polar solvent was added, but then there is a risk of loss of the non-polar analytes which are eluted first in adsorption chromatography. A mixture containing 12 ml of hexane with 120 μ l of isopropanol was selected, which permits good dissolution of the extract without loss of the more apolar targeted pesticides. After conditioning the 500 mg Florisil cartridge with 10 ml of methanol-ethyl acetate (50:50, v/v) and 10 ml of hexane, the extract was applied, dissolved in the hexane-isopropanol solution, the cartridge was air-dried and desorption of the targeted pesticide was obtained with 12 ml of a solution of methanol-ethyl acetate (50:50, v/v). The efficiency of such a clean-up is

extract on Florisil. The detection limits are really improved and are in the range 0.05–0.3 $\mu\text{g/l}$. However, average recoveries were around 10% lower because of losses during the additional clean-up, but were still in the range 70–100%.

4.3.2.2.3. Fractionation by step elution. Silica gel was used in a procedure described for the clean-up of crude extracts from plant material for the determination of pesticide residues [290]. The 500 mg silica cartridges were conditioned by a mixture of acetone and ether (30:70, v/v) and 5 ml of hexane. After application of the extracts dissolved in hexane it was shown that more than 160 pesticides could be fractionated with good recoveries by flushing the silica cartridge with three solutions of increasing polarity, i.e., 5 ml of ether–hexane (20:80, v/v), then 5 ml of ether–hexane (60:40, v/v), and 5 ml of acetone–ether (30:70, v/v) with an efficient clean-up.

Silica gel, Florisil and alumina have been compared for the clean-up of extracts from soil and water from the vadose zone for the determination of organochlorine and organophosphorus pesticides [291]. The results showed that silica was the best choice of adsorbent. Non-polar interferences were removed by a flushing with cyclohexane. A step-elution with 6 ml of hexane containing 5% of ethyl acetate, then 4 ml of ethyl acetate, allowed fractionation of the organochlorine pesticides in the first fraction and then the organophosphorus pesticides in the second one.

4.3.2.3. Clean-up using analytical LC silica columns

The basic principle of this procedure is adsorption or normal phase chromatography, as above, but an analytical LC column is used for the fractionation. This results in more efficient fractionation and more reproducible results, since a UV detector is used to monitor the elution pattern. This was applied to the semi-preparative separation of lipid extracts after saponification, with elution by a mobile phase containing 0.5–10% of 2-propanol in hexane. In a single injection it was possible to separate the lipidic fraction in alkanes, aromatic hydrocarbons, fatty acids, sterols and hydroxy-fatty acids [292]. A similar multiresidue analytical method was described for pesticides, transformation products and related toxicants, based upon the fractionation of the extracted residue on a Partisil silica gel normal phase column followed by selective-detector gas chromatographic determination of the components in each fraction [293]. The LC mobile-phase gradient, i.e., hexane to methyl *t*-butyl ether gave good chromatographic efficiency, resolution, and recovery for 61 test compounds and allowed their collection in four fractions spanning polarities from low-polarity organochlorine compounds in fraction 1 to *N*-methylcarbamates and organophosphorus oxons in fraction 4, as shown in Fig. 4.36. Table 4.19 illustrates the recoveries obtained for 14 pesticides through this LC fractionation.

A similar approach was described for the clean-up of fatty matrices and the fractionation of PCBs and organochlorine pesticides [294,295]. Compared to conventional clean-up methods with cartridges, this method was found to be advantageous because breakthrough of the matrix can be monitored by UV spectrophotometry.

TABLE 4.19

RECOVERIES OF FOURTEEN PESTICIDES THROUGH LC FRACTIONATION ON PARTISIL SILICA GEL, DESCRIBED IN FIG. 4.36 (FROM REF. [293] WITH PERMISSION)

Fraction 1		Fraction 2		Fraction 3		Fraction 4	
Chemical	R (%)	Chemical	R (%)	Chemical	R (%)	Chemical	R (%)
DDT	91	Trifluralin	92	Methiocarb	95	Methomyl	95
Chlorpyrifos	90	Parathion	88	Carbaryl	98		
DDD	89	Me-parathion	92	Terbutol	90		
Ronnel	93	Me-Trithion	88	Carbofuran	98		
Methoxychlor	90						

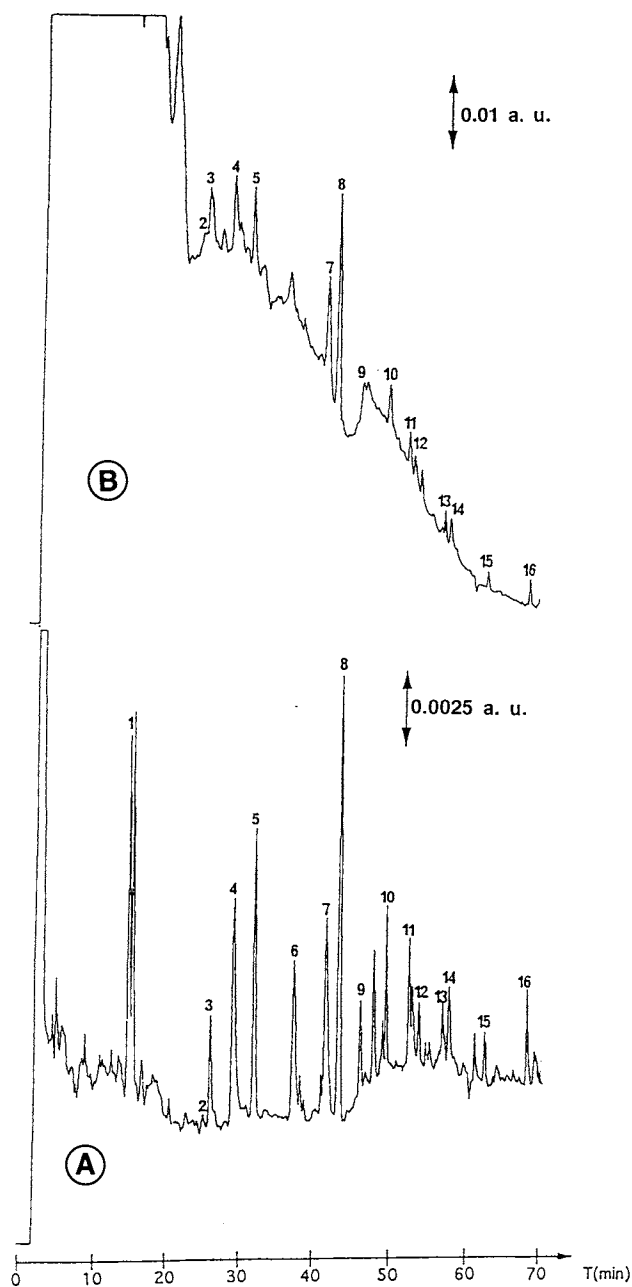
4.3.3. Clean-up using size-exclusion chromatography

4.3.3.1. Basic principles and sorbents

Clean-up using size-exclusion (or gel permeation) chromatography is based on separation by molecular size. Fractionation by polarity using Florisil, silica gel, or alumina selects a limited range of the pesticides but does not remove high molecular weight materials of similar polarity. In contrast, size-exclusion chromatography (SEC) primarily removes materials of high molecular weight, leaving all the pesticides and other compounds of the same weight in the selected fraction. That is particularly important for matrices containing high-molecular-weight interferences such as triglycerides in food or humic substances in soils. In the current environmental analysis of pesticides, polystyrene columns are the most used SEC sorbents, and are eluted with cyclohexane, ethyl acetate–toluene, cyclohexane–dichloromethane, or cyclohexane–ethyl acetate. This last mixture is often selected because of its compatibility with the ethyl acetate used for extraction of pesticides in various solid and liquid matrices [296].

A comparative study was carried out using various types of SEC columns for the isolation of the pesticides monuron, linuron, monolinuron, isoproturon, propanil,

Fig. 4.35. Effect of the clean-up of samples (B) injection of an extract from the River Seine spiked with 0.5 µg/l of each analyte extracted at pH 2 with a C₁₈ cartridge, (A) injection of the same extract after clean-up on a Florisil cartridge From Ref. [114] with permission. (a) Preconcentration of 500 ml via a 500 mg C₁₈ silica cartridge, desorption with 3 ml of methanol, evaporation to dryness, and addition of 500 µl of an acetonitrile–water mixture (20:80, v/v). (b) Preconcentration of 500 ml via a 500 mg C₁₈ silica cartridge, desorption with 3 ml of methanol, evaporation to dryness, dissolution in 12 ml of hexane with 120 µl of isopropanol and clean-up on a 500 mg Florisil cartridge; desorption with 12 ml of methanol–ethyl acetate, evaporation to dryness and addition of 500 µl of an acetonitrile–water mixture (20:80, v/v). Analytical column, Bakerbond Narrow Pore C₁₈ silica, 25 cm × 4.6 mm i.d.; acetonitrile gradient with 0.005 M phosphate buffer at pH 3. UV detection at 220 nm. Peaks: (1) chloridazon; (2) aldicarb; (3) metoxuron; (4) simazine; (5) cyanazine; (6) bentazone; (7) atrazine; (8) carbaryl; (9) isoproturon; (10) difenoxuron; (11) ioxynil; (12) MCPP; (13) 2,4-DB; (14) 2,4,5 TP; (15) metolachlor; (16) dinoterb.



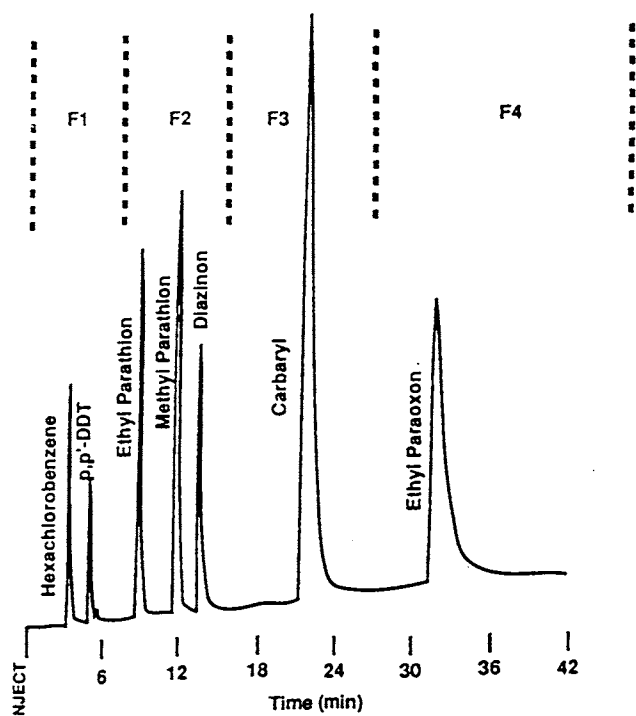


Fig. 4.36. HPLC chromatogram of seven representative pesticides showing zones for fraction collection. Analytical column, Partisil PXS-10/25 silica, $10\ \mu\text{m}$, $25 \times 0.46\ \text{cm}$ i.d., injection of $240\ \mu\text{l}$ of extract dissolved in hexane. Mobile phase, linear gradient from 100% hexane to 100% methyl tert-butyl ether in 30 min, flow rate 1.6 ml/min. From Ref. [293] with permission.

fenitrothion, molinate, alachlor, trifluralin and atrazine from soil samples [297]. Low-resolution SEC polystyrene columns, Bio-Beads SX-3, SX-8 and SX-12, a high-resolution SEC polystyrene column Phenogel, and a silica-based SEC column Zorbax PSM, were compared. The eluent was optimized for the screening of the pesticides and dichloromethane–cyclohexane mixtures gave the best results. The retention times of the various pesticides were measured in order to monitor all the pesticides and eliminate the soil matrix. Figure 4.37A–C gives the SEC profiles obtained with the polystyrene columns and sample collection intervals which correspond to the elution of the targeted pesticides. As expected, the high-resolution Phenogel column is more efficient than the low-resolution Bio-Beads. Figure 4.37D–F shows the liquid chromatograms of the extracts obtained from pesticides added to a soil sample, after clean-up with the SEC columns. No significant differences were found between the Bio-Beads columns, but excellent results were achieved for phenylureas and propanil, with good recoveries using the high-resolution SEC column, with a chromatogram exhibiting no interferences. Similar results were obtained when

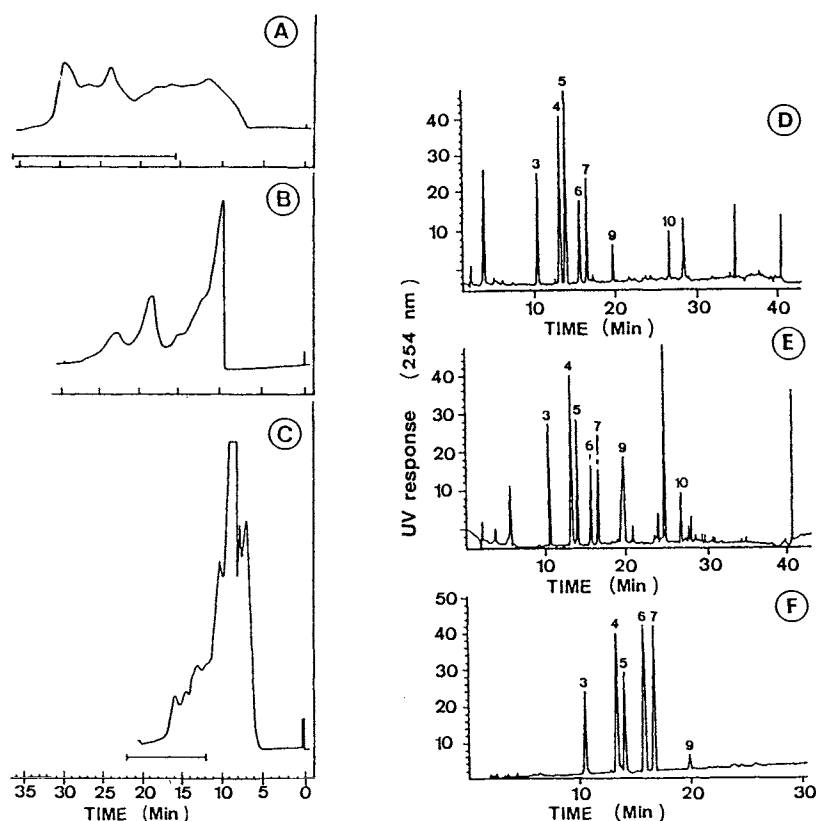


Fig. 4.37. SEC profiles of a soil sample spiked with pesticides (10 $\mu\text{g/g}$ of each) (A) Bio-Beads SX-3; (B) Bio-Beads SX-8; (C) Phenogel high-resolution SEC and reversed-phase chromatograms of the extract after clean-up with (D) Bio-Beads SX-3; (E) Bio-Beads SX-8; (F) Phenogel high-resolution SEC. From Ref. [297]. (a) Eluent, dichloromethane–cyclohexane (1:1, v/v) for Bio-Beads columns and dichloromethane–cyclohexane (1:1, v/v) for Phenogel column. Flow rate, 1 ml/min; UV detection at 254 nm. The sample collection intervals are indicated by the horizontal bars. (b) Zorbax C₈ reversed-phase analytical column eluted with water–methanol–acetonitrile (60:20:20, v/v/v) for 3 min followed by gradient elution to 100% acetonitrile in 30 min at a flow rate of 1 ml/min. Peaks: (3) monuron; (4) isoproturon; (5) monolinuron; (6) linuron; (7) propanil; (8) molinate; (9) alachlor + fenitrothion; (10) trifluralin.

analyzing the extracts by GC-NPD. The silica-based high-resolution SEC also gave good results, thus indicating that high-resolution SEC should be preferred over low-resolution SEC, but the price is three times higher, which can be a serious problem for their implementation in routine analysis.

4.3.3.2. Comparison with methods using polar sorbents

Clean-up procedures using adsorption and size-exclusion chromatography were

compared for the determination of chlorotriazine herbicides and organophosphorus pesticides in soils [298]. The soil samples were soxhlet extracted and the extracts dissolved in 300–500 μ l of *n*-hexane or ethyl acetate, depending on whether the clean-up was carried out on a Florisil column or SEC column. With spiked soils, the recoveries were in the range 75–103% for a mixture of seven pesticides using both clean-up methods. Figure 4.38a represents the profile of a real soil extract after percolation through a Bio-Bead SX-3 column and elution with a mixture of ethyl acetate–cyclohexane (1:1, v/v). The fraction corresponding to elution the organophosphorus and chlorotriazine pesticides was evaporated, and Fig. 4.38b shows the GC-NPD chromatogram of the purified extract. As a comparison, the soil extract was purified using a Florisil column. Figure 4.38c shows the GC-NPD chromatogram. The chromatograms are similar although it can be seen that the Florisil column removes the interferences more efficiently from the soil matrix to give a GC-NPD with a better baseline and fewer unidentified tailing peaks. However, one can say that both clean-up procedures may be efficient when optimized.

4.3.4. Other methods

4.3.4.1. Chemical methods

Chemical treatments with concentrated sulfuric acid, ethanolic potassium hydroxide, and chromium trioxide have been studied for the clean-up of contaminated waters for the determination of organochlorine and organophosphorus pesticides. Viana et al. showed that these treatments destroy some pesticides totally or partially, but left other ones unaltered, and have a clean-up effect so there may be interest in such procedures for some specific compounds [299].

4.3.4.2. Dialysis

The potential of the combination of dialysis, for the removal of interfering humic substances from environmental waters, and trace-enrichment on a precolumn packed with C₁₈ silica for the determination of polar pesticides by LC-UV detection was investigated [300], showing that the chromatograms were improved. The applicability of the dialysis membrane depends on the material used and on the thickness and molecular-weight cut-off value of the membrane. One has to take care to prevent strong interactions of organic analytes with the membrane, and cellulose or cellulose acetate were preferred. Rapid analyte transport through a membrane, and consequently a high recovery per unit time, requires membranes with low thickness and high cut-off value. However, removing humic and fulvic acids requires a small molecular weight cut-off value, so a compromise has to be found. The detection limits in concentration units depends on the configuration of the dialysis system: a high ratio of membrane area/sample volume leads to a high recovery, and a large sample volume to a high response. Detection limits of 1 μ g/l

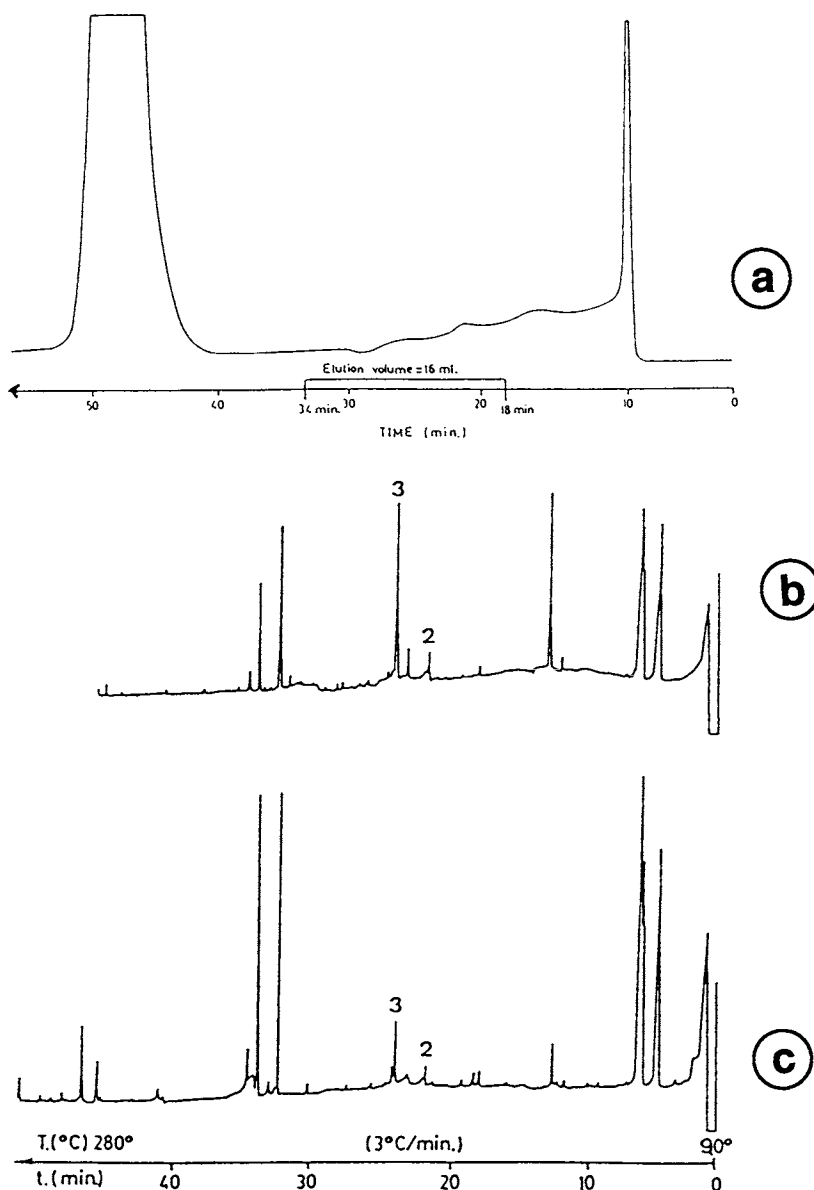


Fig. 4.38. (a) SEC clean-up profile of soil sample and (b,c) comparison of GC-NPD chromatograms of extracts after Florisil clean-up (b) and SEC clean-up (c). From Ref. [298] with permission. (a) Eluent, ethyl acetate-cyclohexane (50:50, v/v) at 1 ml/min. Column: Bio-Beads SX-3 (450 × 10 mm i.d.). Elution volume of organophosphorus and chlorotriazine pesticides between 18 and 34 min. (b) Clean-up using a 150 × 5 mm i.d. Florisil column, extract dissolved in 300–500 μ l hexane, desorption with a mixture of n-hexane-ethyl ether (50:50, v/v) and evaporation to dryness, reconstitution in ethyl acetate before GC injection. (c) Extract corresponding to the 18–34 min fraction evaporated to dryness and reconstituted in ethyl acetate before GC injection. Compounds: (2) deethylatrazine; (3) atrazine at concentrations of 20 and 130 ng/g, respectively.

of six phenylurea herbicides were obtained by using a planar dialysis membrane with a cut-off value of 3.5 kDa and a sample volume of 1.2 ml. These detections were possible using an on-line system which allowed the analysis of all the compounds contained in the sample.

4.4. CONCLUSION AND FURTHER DEVELOPMENTS

Research into new techniques for sample preparation is a very active area. This is partly explained by the need for reducing as much as possible the use, disposal, and release into the environment of toxic solvents, together with a reduction of the total analysis cost. The key words for any development in area of sample preparation are "solvent free method". This is certainly the near end of extensive liquid-liquid extractions followed by Florisil clean-up which are still used in many laboratories. For that purpose, SPE is now becoming a fully accepted technique which allows sample preparation time to be halved or better, and reduces solvent usage by up to 90% compared to liquid-liquid extractions. A proof of its acceptance is the introduction by commercial companies of several new extraction sorbents in both cartridge or disk formats during the past 2 years. Examples are several polymers with high specific surface areas, mixed-mode sulfonated resins, and new carbon-based sorbents. All these sorbents are also more adapted to the extraction of polar pesticides and degradation products. Emerging techniques such as SPME have high potential for eliminating the use of solvents and will certainly be developed with the introduction of new fibres in the near future.

The trend is also to simplify the labour of sample preparation, increasing its reliability, and eliminating the clean-up step needed for aqueous samples by using more selective extraction procedures. These also avoid the problem from interfering components in complex matrices. The development of more selective sorbents, such as immunoaffinity sorbents, is an active research area, because they, too, can be applied for the clean-up of other environmental matrices such as soil, sediments, plants, or food. Increased selectivity can be obtained by coupling different techniques, as was shown in the development of SPE-SFE. Immunoassays are now receiving special attention from analytical laboratories, because the sample-preparation can be avoided or reduced to a minimum with simple SPE or SFE of samples.

The "priority" research developments depend on the regulatory aspects within countries. In Europe, chemists are faced with the drastic drinking water regulatory level of 0.1 $\mu\text{g/l}$ for each pesticide. Therefore, the trend is to the setting up of multiresidue analyses, developing means for avoiding the clean-up step, and the disposal of extraction sorbents for polar pesticides and degradation products. For these last two purposes, the new polymeric extraction sorbents have remarkable potential. In North America, the regulatory levels are higher and for a limited number of pesticides, so it is easier to target the methods for a group of pesticides. On the other hand, the pressure for reduc-

ing the use of organic solvents is much stronger, so active research is being devoted to the development of alternative solvent-free methods.

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On-Line Sample Handling Strategies

5.1. INTRODUCTION

With environmental protection on the agenda of many countries, monitoring programmes for pesticides and degradation products are currently being set up. Consequently, in many laboratories both the number of analyses to be performed and the number of pesticides to be determined have increased rapidly. The interest in using more powerful analytical methods which can be automated and allow the trace determination of many pesticides at the same time has therefore also grown rapidly. In the previous chapter, it was pointed out that sample pretreatment is the weakest link and the primary source of errors and discrepancies between laboratories. The development of more rapid and reliable strategies requires the removal of intermediate steps such as transfers, evaporation and derivatization, i.e., an emphasis on automation and techniques that maximize sample throughput. Solid-phase extraction considerably simplifies the sample pretreatment step, especially when selective sorbents can be used, but there is still some evaporation and transfer of the extract, so that losses from thermal decomposition still occur and contamination risks remain. With the introduction of bridge arms that can automatically transfer SPE collection vials to GC, LC or SFC autosamplers, the automation of the whole analytical scheme, i.e., the SPE process and chromatographic separation, can be performed.

In an on-line system, the sample preparation step and the separation plus detection are combined in one analytical train. In most cases, the sample preparation step is performed by SPE, and the SPE precolumn is directly desorbed into the analytical column. Therefore, on-line SPE is less laborious and time-consuming than off-line SPE, but the most important advantages are that (i) the risk of contamination of the sample and sample extract is greatly reduced, (ii) analyte losses by evaporation do not occur, and (iii) the totality of the extracted species is transferred and analyzed. In contrast to off-line SPE, where only an aliquot of the extract is injected into the chromatograph, for example, in most instances, a volume of 1–5 μl is injected into the GC whereas the extract is dissolved in at least 100 μl , the analysis of the complete sample allows the sample volume to be dramatically reduced to less than 5–

10 ml when SPE is coupled to GC or to 100–150 ml when it is coupled to LC. Finally, very low volumes of solvent are required for the on-line SPE process. The first commercial automated devices became available for the on-line coupling of SPE with LC because of the good compatibility of the LC aqueous mobile phases with the SPE of the samples. On-line coupling of SPE with GC is more delicate because of the inherent incompatibility between the aqueous part of the SPE step and the dry part of the GC system. However, much work has been done in this area and automated devices have recently become available. On-line techniques which couple SPE with the various chromatographic modes offer a fast, modern and reliable approach for monitoring traces of pesticides in water using completely automated methods with no risk of loss or contamination since there is no sample manipulation between the sample percolation and the analysis.

It is clear that the state-of-the-art depends on the on-line methodology. The on-line SPE-LC-DAD system is being used routinely in many laboratories; this is the subject of the first part of the chapter. Most of the multiresidue methods for polar pesticides or degradation products involve LC, with identification of compounds using a diode-array UV detector (DAD). Special attention is given to the sample volume that can be handled, depending on the nature of the sorbent packed in the precolumn, and on the polarity of the analytes. The corresponding detection limits that can be reached in real samples, i.e., drinking, surface or sea water are presented. The total approach of the system is discussed. In the event of insufficient sensitivity, the use of more selective sorbents, or of on-line post-column derivatization techniques is required; these are described. Confirmation of identities is often obtained by mass spectrometry (MS) techniques which can also be included in the on-line system. The on-line coupling with MS can be added to on-line SPE-LC systems, thus providing the most efficient arrangement for multiresidue analysis of pesticides.

The second part of the chapter is devoted to the on-line coupling of SPE with GC. The removal of water, required before transfer to the GC column, is described and various applications using different detection modes are presented. The new methods involving solid-phase microextraction have been discussed in the previous chapter. Although it can be considered as an on-line integrated technique since it includes the direct injection of the species concentrated on the fibre into the GC, there are no special conditions linked to the on-line transfer of the species from the fibre to the GC.

In the last part of this chapter, the potential of the on-line coupling of SFE with SFC is presented.

5.2. ON-LINE TECHNIQUES WITH SEPARATION BY LIQUID CHROMATOGRAPHY

As seen in Chapter 3, liquid chromatography (LC) is particularly well adapted to multiresidue analysis because of its suitability for direct analysis over a wide range of polarity, without risk of thermal decomposition. The basic principles of SPE that

have been described in the previous chapter are valuable for the prediction of recoveries. The main differences lie in the selection of the sorbent and in the possibility of coupling several sorbents in series.

5.2.1. On-line solid-phase extraction and liquid chromatography with conventional detectors (SPE-LC systems)

5.2.1.1. On-line arrangements

The on-line coupling of SPE to LC is particularly easy to perform in any laboratory, and has been described extensively in general reviews dealing with the on-line preconcentration of organic compounds in environmental or biological samples [1–19]. The SPE column, the so-called precolumn, is generally made of stainless steel in order to be pressure-resistant. The apparatus is represented in Fig. 5.1. The trace-enrichment is carried out on a small precolumn which is placed at the sample-loop position of a six-port liquid switching valve. A solvent delivery unit (SDU) provides the solvents necessary to purge, wash, and activate the precolumn. After conditioning, sample application, and eventual clean-up via the pump of the SPE part, the precolumn is placed in front of an analytical column by switching the valve into the “inject” position. The trapped compounds are then eluted directly from the precolumn into the analytical column by a suitable mobile phase which also brings about the chromatographic separation. Quantitative results of good accuracy can be expected as there is no sample manipulation between the preconcentration and the analysis. In contrast with off-line SPE procedures, the entire sample is transferred and analyzed, which allows the handling of smaller sample volumes. The LC system is often run in the reversed-phase mode, using apolar alkyl silica analytical columns because the mobile phase is a partly aqueous solvent mixture. This means that residual water in the precolumn after the preconcentration of aqueous samples does not have to be removed before the desorption. The addition of a second switching valve allows both direct injection onto the analytical column and preconcentration via the precolumn. Automation is very easy and several devices are now commercially available (such as the Prospekt from Spark Holland, and OSP-2 from Merck). In these systems, a new disposable precolumn is used for each run and the exchange is automatic. The whole sequence can be programmed and can be performed on a sample whilst the on-line analysis of a previous sample occurs [20–29]. This full automation has been used for on-site monitoring of pesticides in surface waters as part of an early warning alarm system [18,22,28,29]. These studies have contributed greatly in demonstrating that on-line SPE-LC is a robust and reliable technique that can be applied routinely in the field.

5.2.1.2. Precolumns

The size of the precolumn is an important parameter because the profile of the concentrated species transferred from the precolumn to the analytical column should

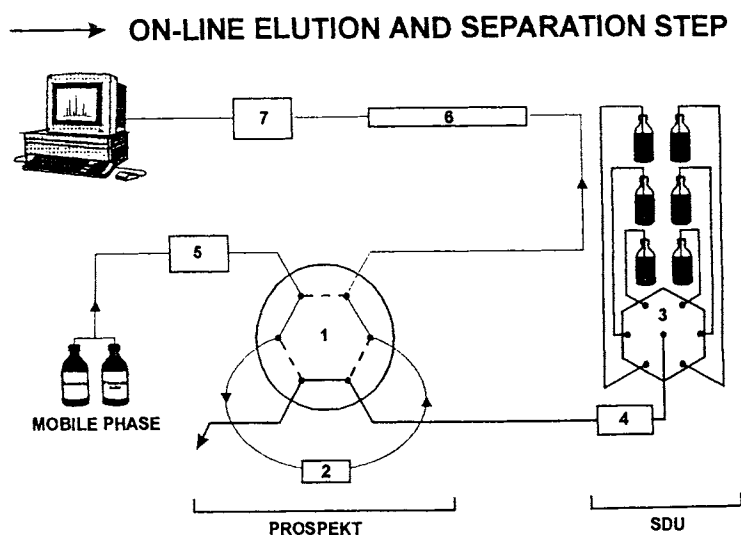
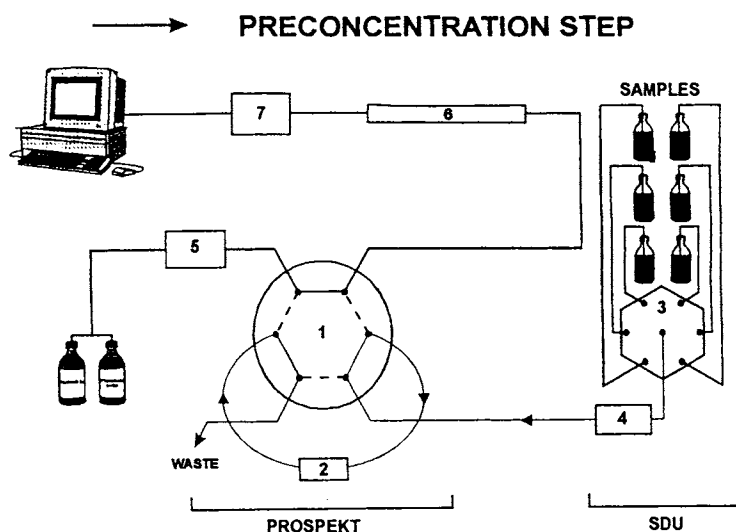


Fig. 5.1. On-line arrangement. 1, LC switching valve; 2, precolumn; 3, switching valve of the solvent delivery unit; 4, pre-concentration pump; 5, LC pump; 6, analytical column; 7, detector.

be as narrow as possible at the beginning of the separation in order to avoid band-broadening. The quality of the coupling can easily be controlled by comparing chromatograms obtained by direct injection with those obtained by on-line preconcentration via the precolumn. The dimensions of the precolumn should be adapted to those of the analytical column [30,31] and are typically 2–15 mm long and 1–4.6 mm i.d. for a classical 15–25 cm long analytical column. The use of a

small precolumn is important when the separation is carried out with an isocratic mobile phase [32]. It was shown that the dimensions of the precolumn can be increased without band-broadening when an appropriate methanol or acetonitrile gradient was applied for the separation [33].

Although it was first recommended to pack precolumns with 5–10 μm packings [4], the trend now is to use 15–40 μm packings in order to have a high sampling rate during the loading of the sample and to prevent clogging with, for example, a surface water sample. Despite the granulometry, it is recommended to use LC-grade- and pressure-resistant sorbents. Prepacked precolumns with different sorbents are now available from various manufacturers. It is also easy to pack a precolumn in the laboratory, thus allowing the potential of using new sorbents.

The use of Empore disks in on-line applications became possible after the production by U.A.Th. Brinkman's group of an appropriate membrane-disk holder [23,34–37]. Here again the advantage is the possibility of higher flow rates during the sample loading.

Backflush-desorption of the precolumn should give the least amount of extra band broadening and should provide better peak shapes than forward-desorption. However, backflush-desorption has the drawback of creating problems of clogging of the analytical column when real samples are used. In the forward desorption mode, the precolumn has the additional role of acting as a guard column and thus preserving the lifetime of the analytical column. With precolumns of small dimensions, our own experience has indicated that very little difference can be observed between backflush and forward desorption.

The compatibility between the sorbent and the precolumn is important. The most efficient system is ideally obtained for a precolumn and an analytical column of the same nature [4]. Various sorbents can be packed in precolumns which have different abilities for trapping the compounds, especially the more polar ones, but in practice it appears that the choice of sorbent to be packed in the precolumn is greater than the choice of analytical columns. When many components are to be separated, over a large range of polarity, their separation requires a highly efficient analytical column with both water-rich and organic-rich mobile phases. At present, only C_{18} silica columns meet this requirement. Polymeric SDB or carbonaceous columns are very efficient in organic-rich mobile phases but much less so in water-rich ones. The compatibility of sorbents for precolumn and analytical columns is discussed for each precolumn sorbent.

5.2.1.3. Selection of the sorbent for the precolumn

A serious limitation of SPE-LC systems is that they use small precolumns which therefore contain a small amount of sorbent. In contrast to off-line SPE where there is the possibility of increasing the breakthrough volumes, V_b , by increasing the amount of sorbent in the cartridge, in on-line SPE, when compounds are poorly retained the only solution is to select a more retentive sorbent. The basic principles of

SPE are similar for off-line and on-line methods and have been extensively described in the previous chapter.

5.2.1.3.1. *Non-selective sorbents*

Alkyl bonded silica. Most of the published off-line procedures now utilize disposable cartridges packed with C_8 or C_{18} silica and this is certainly the first sorbent to be tried in on-line techniques. The average limits of detection (LOD) with UV-DAD are between 0.5 and 10 ng injected, depending on the pesticide's UV properties. For an average LOD of 5 ng, if one wants determination in water at a concentration of 1 $\mu\text{g/l}$, a sample volume of 5 ml will be sufficient. Reupert et al. [27] have shown that the on-line handling of a 5 ml river-water sample was enough to detect most pesticides at the 5 $\mu\text{g/l}$ level. However, 5 ml will usually be too small a volume to reach a concentration limit of detection in real water samples and the presence of humic substances and other contaminants will often require the handling of a 10-fold higher volume, as shown below. When concentration levels of 50 ng/l are required, the sample volume should be increased to at least 100 ml.

Among alkyl silica sorbents, several C_8 and C_{18} formulations are available in addition to cyclohexyl and phenylsilicas. We know from LC data that C_8 silicas usually provide lower $\log k'_w$ values (provided that C_{18} sorbents with high carbon contents were considered for comparison). For this reason, the use of a precolumn packed with C_8 silica will lead to lower V_b values and is not recommended for the more polar pesticides. Table 5.1 summarizes the percent recoveries using on-line cartridges packed with different alkyl silicas and with the apolar copolymer PLRP-S. The sample volume was 200 ml. Recoveries are very low with a C_2 silica precolumn and are lower with a C_8 than with a C_{18} silica precolumn [38]. Similar results were obtained when comparing V_b values using C_{18} silicas, characterized by 5 and 15% carbon content and C_8 silica for the preconcentration of a mixture of phenylureas and triazine herbicides [39]. This is also illustrated in Fig. 5.2 where the same volumes of water spiked with 24 polar and moderately polar pesticides and degradation products were analyzed on-line using a 15×3 mm i.d. precolumn packed using C_8 silica (Fig. 5.2a), cyclohexyl silica (Fig. 5.2b) and C_{18} silica (Fig. 5.2c). For the first-eluted pesticides, the peak heights obtained by C_8 or cyclohexyl silica are lower than those obtained with C_{18} silica, thus showing lower recoveries. On the other hand, the amount of co-concentrated species is lower on C_8 silica. With the C_{18} silica there is a large peak of interfering material eluted during the first 20 min of the chromatogram. This result is consistent with those reported in Ref. [39], showing the problem encountered in determination of polar pesticides requiring sorbents that also concentrate effectively the humic material of surface waters.

One has to take care that C_{18} sorbents "specific for the trapping of polar pesticides" do not really extract the polar compounds "selectively", as is often mentioned. The breakthrough volumes have been measured for polar carbamates by measuring the recoveries obtained with increasing sample volumes, using disposable cartridges

TABLE 5.1
PERCENT RECOVERY OF PESTICIDES USING CARTRIDGES PACKED WITH DIFFERENT
PHASE MATERIAL

Compound	Phase			
	C-2	C-8	C-18	PLRP-S
Hydroxyatrazine	0	10	20	47
De-isopropylatrazine	0	15	30	55
Desethylatrazine	0	20	32	60
Hexazinone	<5	50	85	95
Metoxuron	<5	20	56	95
Simazine	<5	25	60	97
Monuron	<5	10	35	98
Cyanazine	<5	60	90	98
Methabenzthiazuron	8	76	88	96
Chlortoluron	8	45	80	97
Atrazine	13	75	85	97
Isoproturon	10	75	81	92
Diuron	12	50	75	95
Metobromuron	13	35	75	97
Metazachlor	15	40	90	98
Sebutylazine	17	60	90	97
Terbutylazine	18	70	90	97
Chloroxuron	15	75	87	95
Linuron	13	80	89	95
Anilazine	15	90	92	97
Metolachlor	40	80	89	95
Neburon	50	85	90	97

Sample volume 200 ml, precolumn size: 10 × 2 mm i.d.. From Ref. [38].

of the Prospekt system packed with two conventional C₁₈ sorbents from two different manufacturers. One of these was a C₁₈ sorbent, specific for polar compounds, C₁₈OH, and the other was PLRP-S SDB sorbent [40]. The results are given in Table 5.2 and show that the recoveries, and consequently breakthrough volumes, are slightly lower for C₁₈OH sorbent than for the two conventional C₁₈ sorbents for which the recoveries are very similar for all the sample volumes. These results are expected since the C₁₈ sorbents for "polar" analytes contain polar moieties in order to provide better contact of solutes with the sorbent, but some of them can have lower carbon contents so that they provide slightly lower retention of all the analytes. It is worthwhile to note that the PLRP-S sorbent provides better recoveries, as expected, although breakthrough occurs for 10–20 ml for the more polar aldicarb sulfone, ox-amyl, and methomyl.

Empore disks enmeshed with C₁₈ silica have also been used in precolumns. Breakthrough volumes of 4, 110 and 150 ml were measured for aldicarb sulfone,

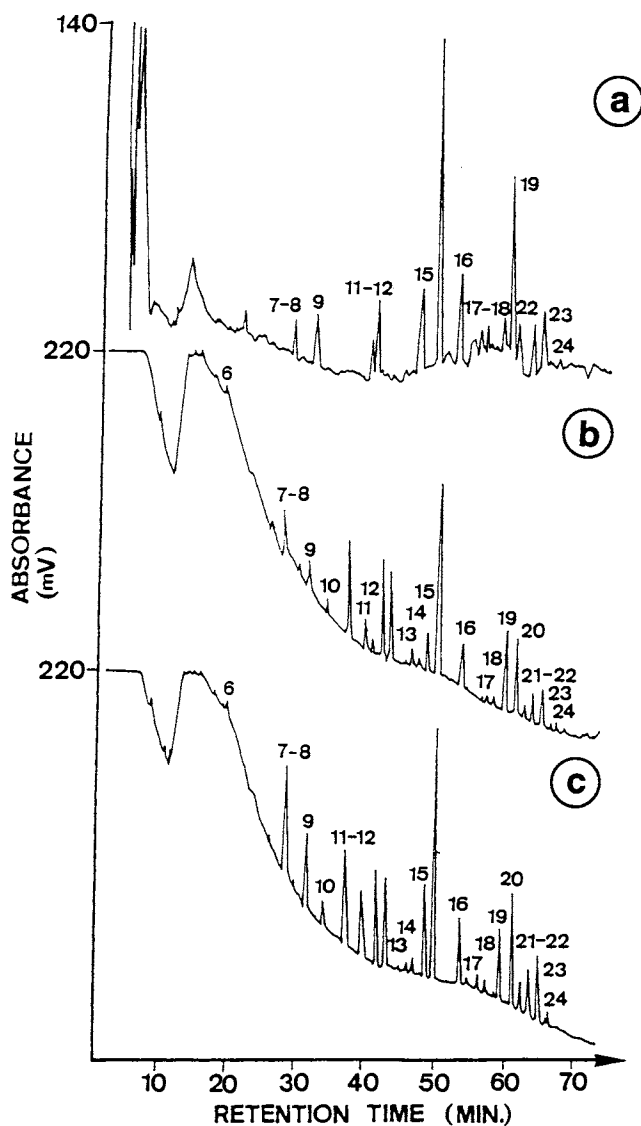


Fig. 5.2. Effect of the nature of the alkyl bonded silica packed in the precolumn for the on-line analysis of 150 ml of Ebro river water spiked with $0.3 \mu\text{g/l}$ of a mixture of 24 polar pesticides. From Ref. [1]. Precolumns $1.5 \times 0.3 \text{ cm i.d.}$; packed with (a) C₈ silica, (b) cyclohexyl silica and (c) C₁₈ silica (C₁₈ Polar Plus). Analytical column, LichroCart cartridge column ($25 \times 0.46 \text{ cm i.d.}$) packed with $4 \mu\text{m}$ Supersphere 60 RP-8 from Merck. UV detection at 220 nm. Peak number (6) de-isopropylatrazine, (7) 3-hydroxycarbofuran, (8) methiocarb sulfoxide, (9) de-ethylatrazine, (10) methiocarb sulfone, (11) 3-ketocarbofuranphenol, (12) butoxycarboxim, (13) aldicarb, (14) 3-ketocarbofuran, (15) 8-hydroxybentazone, (16) simazine, (17) baygon, (18) carbofuran, (19) bentazone, (20) carbaryl, (21) chlortoluron, (22) MCPA, (23) atrazine, (24) isoproturon.

TABLE 5.2
COMPARISON OF RECOVERIES OBTAINED FOR POLAR CARBAMATES WITH FOUR DIFFERENT SORBENTS IN THE PRECOLUMNS (TWO CLASSICAL C₁₈ SILICAS, ONE C₁₈ SILICA SPECIFIC FOR POLAR COMPOUNDS, AND PLRP-S) WITH INCREASING SAMPLE VOLUMES

Compounds	Sample volume (ml)																			
	5				10				25				50				100			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Aldicarb sulfone	39	71	68	100	19	39	31	81	9	18	16	29	4	8	9	22	1	3	3	10
Oxamyl	50	79	77	100	25	49	42	95	12	25	22	49	6	13	11	36	3	6	5	17
Methomyl	39	72	66	101	18	35	29	86	9	19	16	39	4	9	7	27	2	4	3	13
Aldicarb	95	90	90	100	90	99	100	92	50	102	97	89	24	71	70	98	12	36	33	94
Carbofuran	100	93	95	99	96	97	95	100	102	98	106	94	74	103	96	102	43	92	90	99
Carbaryl	98	92	94	98	98	99	99	98	106	106	104	94	75	103	99	101	41	93	94	101
Methiocarb	101	92	99	102	97	100	100	97	107	110	110	98	107	108	108	106	104	104	104	105

Precolumn size: 10 × 2 mm i.d. (a) C₁₈OH from Analytichem; (b) C₁₈ sorbent from J.T. Baker; (c) C₁₈ sorbent from Analytichem; and (d) PLRP-S SDB.

aldicarb and metolachlor when using the precolumn packed with ten layers of C_{18} disks [35].

In this section, we have pointed out the limitation of using C_{18} silica in conventional small on-line precolumns for the preconcentration of the more polar pesticides. One has to be aware of this limitation when carrying out multiresidue analysis. The classification of pesticides by increasing hydrophobicity is a great help in rapidly estimating whether C_{18} will be the appropriate sorbent, depending on the trace-level required. Another limitation lies in the pH range that should be between 2–3 and 8.

Apolar copolymers. The porous SDB sorbents that are pressure resistant and easily available for liquid chromatography are mainly the PRP-1 from Hamilton and the PLRP-S from Polymer Laboratories. Some LC-generated data obtained using PRP-1 have indicated that the k'_w values were between 25 and 40 times higher than k'_w obtained with C_{18} silica [33]. Table 5.3 reports comparisons of V_b values obtained on precolumns packed respectively with C_{18} silica and PLRP-S copolymers. Tables 5.1 and 5.2 also allowed a comparison of the percent recoveries obtained with alkylsilicas and PLRP-S. Except for polar carbamates and polar metabolites of atrazine, i.e., de-isopropyl-, de-ethyl- and hydroxy-atrazine, all the recoveries are higher than 90–95% with the handling of 200 ml. The increase in V_b values for the more polar pesticides explains why most on-line multiresidue pesticide analyses are performed with PLRP-S or PRP-1 prepacked precolumns. Figure 5.3 shows the chromatograms corresponding to the on-line analysis of 150 ml of a drinking water sample, non-spiked in Fig. 5.3a and spiked with $0.1 \mu\text{g/l}$ of a mixture containing 21 pesticides with a wide range of polarities in Fig. 5.3b. Although breakthrough has occurred for de-ethylatrazine, there is no problem in identifying de-ethylatrazine at a concentration of

TABLE 5.3

BREAKTHROUGH VOLUMES IN ml: (A) CALCULATED FOR A 1×0.2 cm i.d. PRECOLUMN PACKED WITH C_{18} SILICA, AND (B) MEASURED ON A 1×0.2 cm i.d. PRECOLUMN PACKED WITH PLRP-S

Compound	C_{18}	PLRP-S
Oxamyl	4 ± 1	30 ± 5
DIA	2 ± 1	25 ± 10
Metamitron	3 ± 1	75 ± 10
Chloridazon	2 ± 1	90 ± 20
Carbendazim	4 ± 1	180 ± 20
Aldicarb	6 ± 1	250 ± 30
Simazine	23 ± 5	>350
Metribuzin	26 ± 6	>350
Carbofuran	32 ± 8	>350
Atrazine	80 ± 2	>350
Isoproturon	80 ± 20	>350
Terbuthylazine	130 ± 30	>350

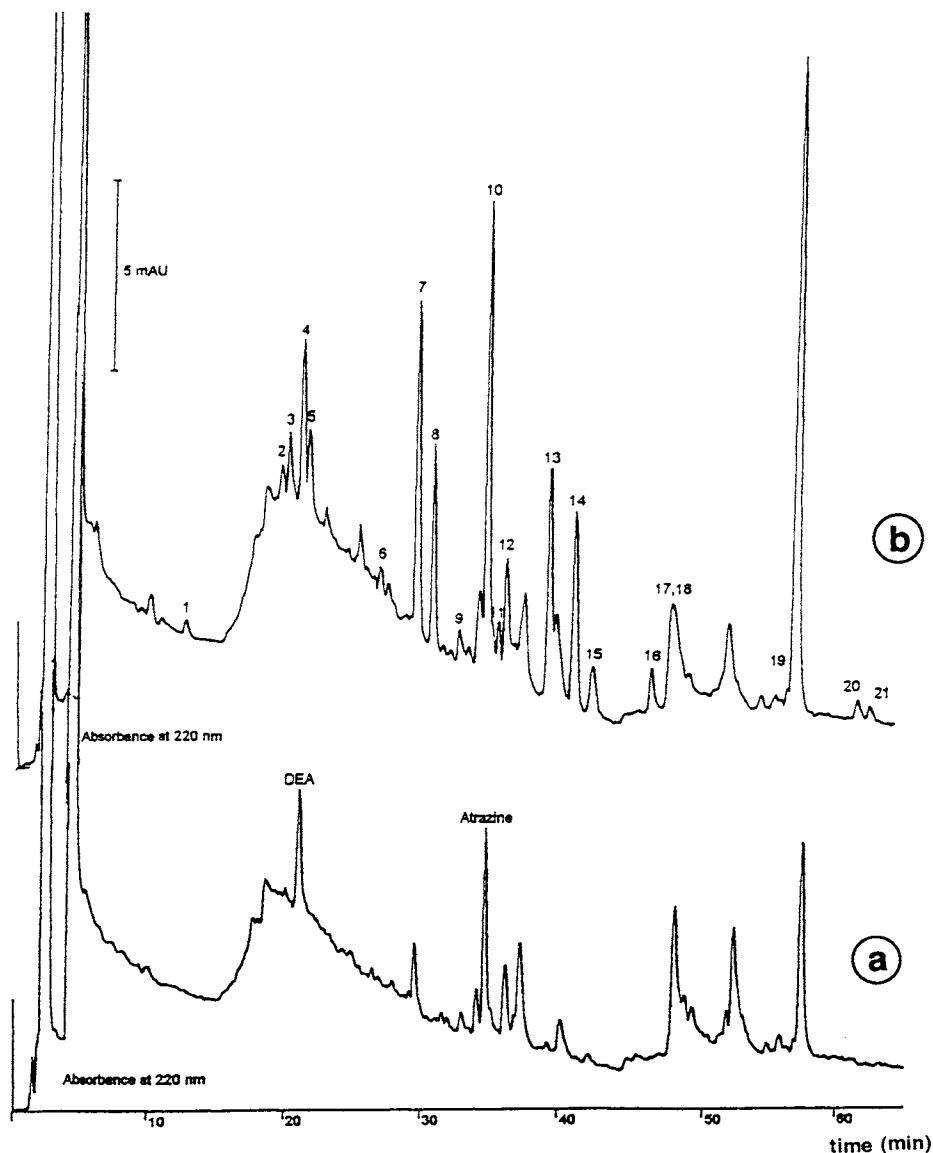


Fig. 5.3. On-line analysis of (a) 150 ml of a drinking-water sample, and (b) 150 ml of the same sample spiked with $0.1 \mu\text{g/l}$ of a mixture of 21 pesticides using the Prospekt system. Precolumn, $1 \times 0.2 \text{ cm}$ i.d. packed with PLRP-S from Polymer Laboratories; C_{18} analytical column TSK ODS-80TM, $25 \times 0.46 \text{ cm}$ i.d., water-acetonitrile gradient; 15% ACN from 0 to 10 min, 50% at 33 min, 50% at 40 min, 70% at 70 min; UV DAD detection, chromatogram at 220 nm. Peak numbers: (1) de-isopropylatrazine; (2) metamitron; (3) hydroxyatrazine; (4) de-ethylatrazine; (5) chloridazon; (6) aldicarb; (7) simazine; (8) de-ethylterbutylazine; (9) carbofuran; (10) atrazine; (11) isoproturon; (12) diuron; (13) propazine; (14) terbutylazine; (15) linuron; (16) terbuconazole; (17) alachlor; (18) metolachlor; (19) fenoxaprop-*p*-ethyl; (20) pendimethalin; (21) trifluralin.

$0.09 \pm 0.01 \mu\text{g/l}$ in such waters. De-isopropylatrazine should be difficult to determine at the $0.1 \mu\text{g/l}$ level, owing to its too early breakthrough. Another advantage of copolymer sorbents over C_{18} silica is that they can be used in the pH range 1–13.

Since the analytes are retained more by the sorbent of the precolumn than by the analytical column, band broadening can occur because the compounds may not re-concentrate in a narrow enough band at the top of the analytical column. This problem does not usually arise, especially when an acetonitrile gradient is used. An example is shown in Fig. 5.4 which shows the results from on-line coupling using a C_{18} and a PLRP-S precolumn for the determination of several phenylureas over a large range of polarity [41]. No difference can be detected in the peak shapes when a C_{18} or a PLRP-S precolumn is coupled to the C_{18} analytical column. Only the peak heights are different as a result of breakthrough of the compounds first eluted from the C_{18} precolumns. The importance of using an acetonitrile gradient instead of a methanol gradient when very polar analytes are analyzed is shown in Fig. 5.5 for the on-line analysis of carbamates [40]. When the separation is performed with a methanol gradient, band broadening occurs if a PLRP-S precolumn is used for the preconcentration (Fig. 5.5b) but it is not observed with the C_{18}OH precolumn (Fig. 5.5a). In the separation using an acetonitrile gradient, no band broadening is observed with either the C_{18}OH or PLRP-S precolumn, as shown in Fig. 5.5c,d. This is explained by the higher eluting power of acetonitrile (compared to methanol) which desorbs the analytes more rapidly, thus providing a better re-concentration effect on top of the analytical column. It can also be seen by comparing the heights of peaks 1–3 in Fig. 5.5 that the recoveries obtained with the C_{18}OH precolumn (Fig. 5.5a,c) are lower than those obtained with the PLRP-S precolumn (Fig. 5.5b,d).

When pesticides over a wide range of polarity are analyzed, on-line losses can occur for the more polar ones, as a result of breakthrough, but losses can also occur for the more apolar pesticides as a result of adsorption in connective tubing and sample bottles, as in off-line methods. This problem was encountered in the screening of organophosphorus pesticides [42]. This class of compounds is often analyzed by GC but some compounds do not display good thermostability, so there is interest in their screening using LC-DAD. With the handling of 100 ml samples and using a PLR-S precolumn, the authors have noted poor recoveries for the more polar compounds such as monocrotophos (15%) and vamidothion (70%), excellent recoveries for the medium polarity compounds and recoveries around 80% for the more apolar carbo-phenthion and bromophos ethyl. It was shown that the lower recoveries for the latter group reflected adsorption problems in the inner walls of the connective tubing and the seals of the switching valves of the preconcentration system. This underlines the inherent problem in analyzing compounds over too large a polarity range because adsorption can be minimized only by adding a surfactant or an organic solvent which has the effect of increasing the losses of the more polar compounds.

As described in the previous chapter there is interest in using the new SDB poly-

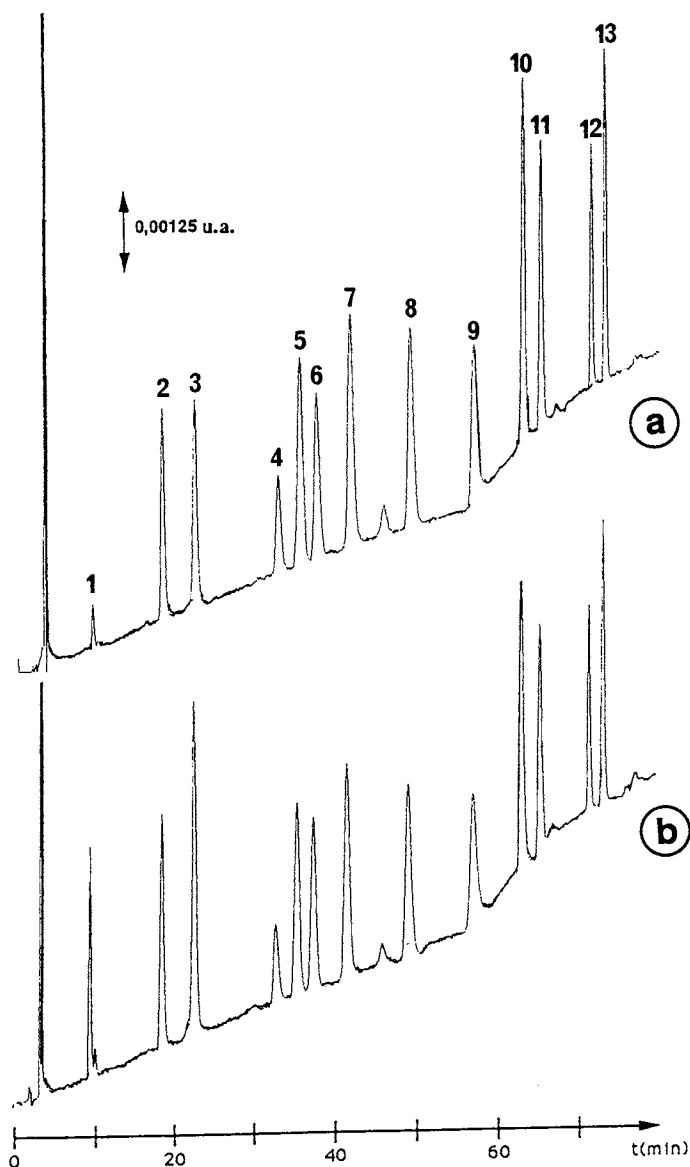


Fig. 5.4. Compatibility between the sorbent in the precolumn and that of the analytical column. Precolumns, 10×2 mm i.d. packed with (a) C_{18} silica and (b) PLRP-S coupled to a C_{18} analytical column, 25×0.46 cm i.d. (Supelcosil LC-18-DB). From Ref. [41]. On-line preconcentration of 100 ml of LC-water spiked with $0.5 \mu\text{g/l}$ of each analyte, using a water-acetonitrile gradient. Solutes: (1) fenuron; (2) metoxuron; (3) monuron; (4) methabenthiuron; (5) chlorotoluron; (6) fluometuron; (7) monolinuron; (8) isoproturon; (9) diuron; (10) difenoxuron; (11) buturon, (12) linuron; (13) chlorbromuron; (14) chloroxuron, (15) diflufenbuzuron, (16) neburon.

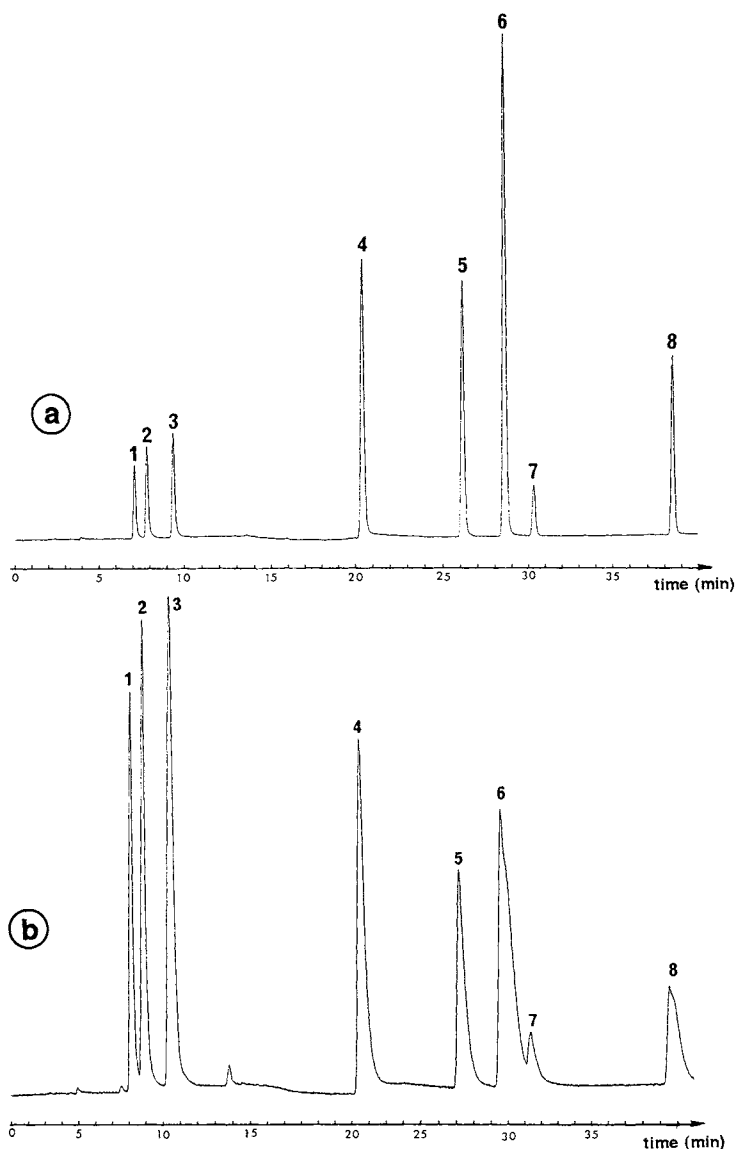


Fig. 5.5. Effect of the gradient of the mobile phase applied for the analytical separation with C_{18} and PLRP-S in the precolumn. (a) $C_{18}OH$ and (b) PLRP-S precolumns in combination with a C_{18} analytical column and a water-methanol gradient. (c) $C_{18}OH$ and (d) PLRP-S precolumns in combination with the same analytical column, but with a water-acetonitrile gradient. On-line preconcentration of 10 ml of LC-grade water spiked with eight carbamates and degradation products with a 10×2 mm i.d. precolumn. Analytical column, TSK ODS-80TM, 25×4.6 mm i.d. FOR (a) and (b), water-methanol gradient, 25% methanol from 0 to 1 min, 34% at 8 min, and 88% at 35 min; (c) and (d), acetonitrile-water gradient, 10–20% from 0 to 15 min and 60% from 25 to 35 min. Flow rate, 1 ml/min. Fluorescence detection with on-line post-column reaction. Solutes: (1) aldicarb sulfone, (2) oxamyl, (3) methomyl, (4) aldicarb, (5) carbofuran, (6) carbaryl, (7) thiofanox, (8) methiocarb.

mer with high specific areas (HSA/SDB) for retaining the more polar analytes which are not retained sufficiently by PLRP-S for detection at the $0.1 \mu\text{g/l}$ level. Table 5.4 shows the higher recoveries which are obtained with a HSA/SDB in comparison to PLRP-S, using precolumns of the same size with a 100 ml sample volume [43].

The problem with this development is that the on-line coupling with a C_{18} analytical column is difficult for on-line analysis of very polar analytes, even with water-acetonitrile gradients, because the difference in retention by the two sorbents is too

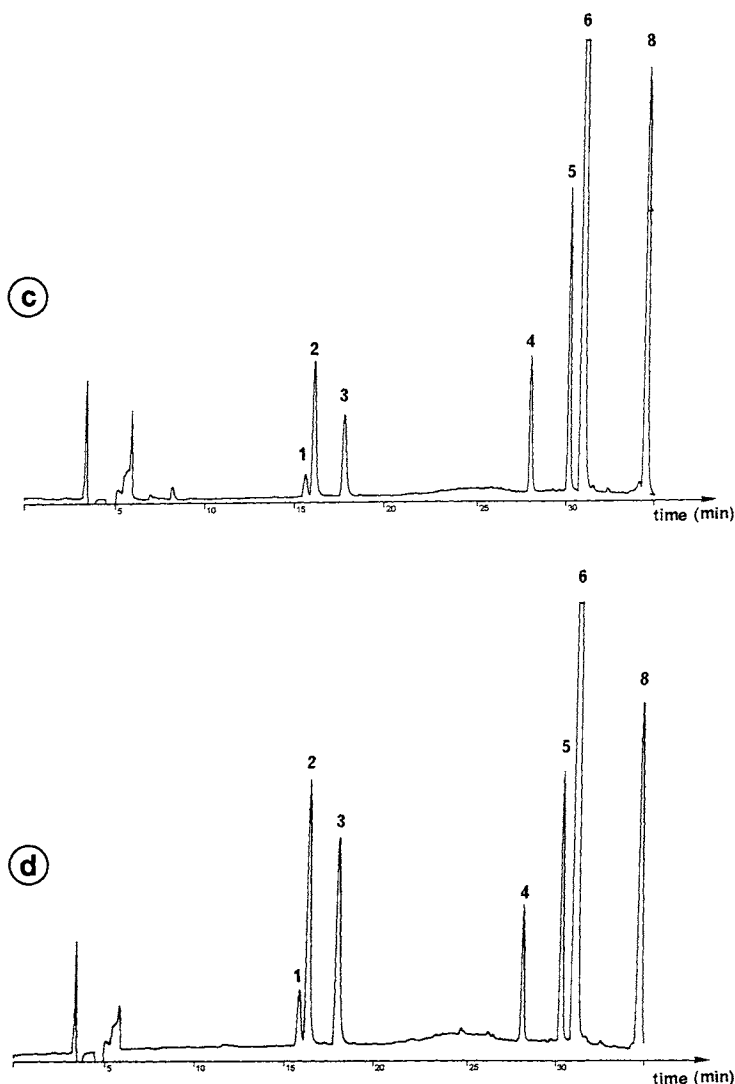


Fig. 5c,d.

large. This is illustrated in Fig. 5.6 which shows the different profiles of the on-line chromatograms with a PLRP-S precolumn (Fig. 5.6a), a SDB precolumn (Fig. 5.6b), and a precolumn packed with porous graphitic carbon (PGC). Large band broadening is observed for SDB just because polar analytes can only be separated on C_{18} columns by using mobile phases which contain a high proportion of water but which are unable to desorb the polar analytes trapped on the HSA/SDB precolumn. This is a limitation of the on-line integration of precolumns packed with HSA/SDB together with C_{18} analytical columns. However, it should be mentioned that on-line coupling of a HSA/SDB precolumn and separation with a C_{18} analytical column is possible, provided that a third pump adds some water after the on-line desorption.

Other columns have been tried to solve the problem discussed above [43]. Analytical columns capable of retaining polar compounds more strongly are required for a simple on-line coupling with an HSA/SDB precolumn. Polymeric analytical columns were tested. First, this type of column is efficient with mobile phases containing a high proportion of organic solvent, but the efficiency decreases dramatically when the mobile phase contains a large proportion of water, as required for the separation of polar analytes. Recent commercially available polymeric columns were shown to be more efficient, but their specific area is too low and consequently, for very polar compounds, retention factors are very similar to those obtained with C_{18} silica columns. Among other possible columns, PGC analytical columns have been shown to achieve some efficient separations of polar analytes. Figure 5.7 shows the pleasing chromatograms obtained with the on-line preconcentration of a HSA/SDB precolumn and a PGC analytical column for the determination of eight polar pesticides at the $0.1 \mu\text{g/l}$ level in drinking water and at the $1 \mu\text{g/l}$ level in surface water.

TABLE 5.4

COMPARISON OF RECOVERIES (%) FOR POLAR PESTICIDES USING PRECOLUMN PACKED WITH LOW- AND HIGH-SPECIFIC AREA COPOLYMER. FROM REF. [43]

	PLRP-S precolumn ^a		SDB precolumn ^b
	50 ml ^c	100 ml ^c	100 ml ^c
Clopyralid	27 ± 3	13 ± 2	97 ± 2
Oxamyl	39 ± 2	14 ± 2	99 ± 3
Dicamba	100 ± 3	93 ± 3	95 ± 1
Monocrotophos	31 ± 4	12 ± 3	98 ± 4
Picloram	92 ± 4	35 ± 3	97 ± 3
Bentazone	101 ± 3	95 ± 2	94 ± 3
De-ethylatrazine	45 ± 3	14 ± 1	97 ± 3
Fenuron	96 ± 4	47 ± 3	100 ± 4

^aPLRS-S precolumn, 8×2 mm i.d.

^bSDB precolumn (HSA/SDB from JT Baker), 8×2 mm i.d.

^cSample volume.

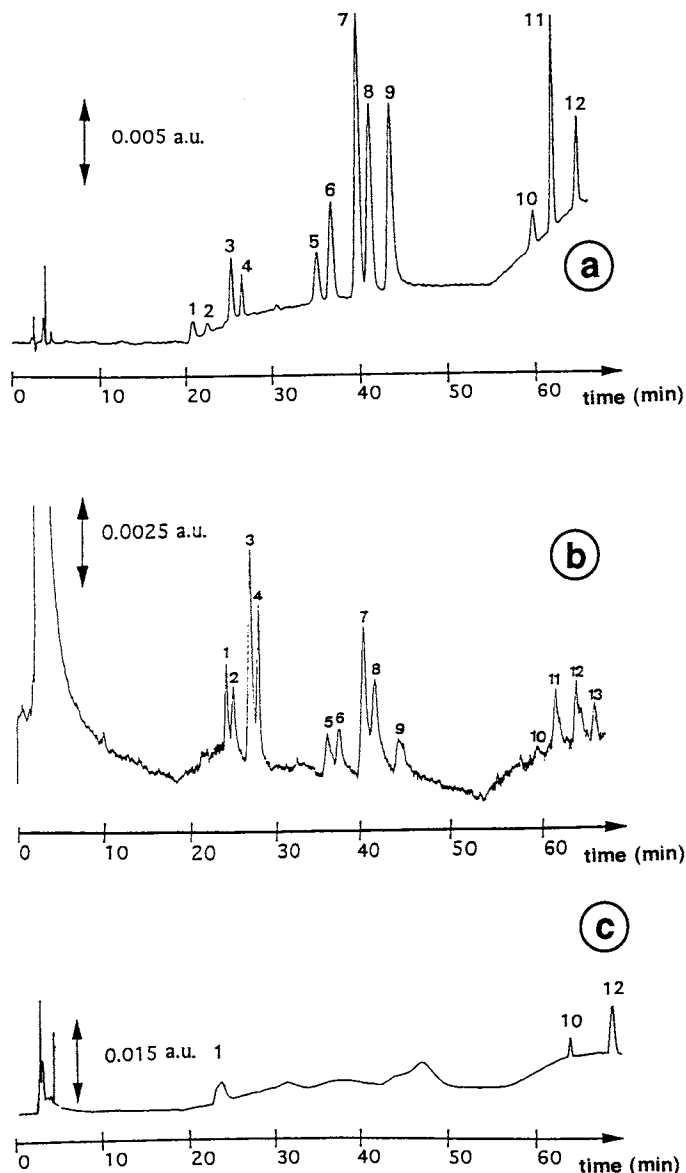


Fig. 5.6. Effect of the sorbent in the precolumn for the on-line coupling with a C_{18} silica analytical column. (a) PLRP-S precolumn, (b) HSA/SDB precolumn, (c) PGC precolumn. From Ref. [44] with permission. On-line preconcentration of 100 ml of water spiked with thirteen polar pesticides and degradation products spiked with $1.5 \mu\text{g/l}$ of each analytes; C_{18} analytical column, Supelcosil LC-18-DB ($25 \times 0.46 \text{ cm i.d.}$), acetonitrile gradient with a $5 \times 10^{-3} \text{ M}$ phosphate buffer at pH 7, 5% acetonitrile from 0 to 15 min, 10% at 20 min, 15% from 40 min to 50 min, 30% at 60 min, and 65% at 65 min. Flow rate 1 ml/min; UV detection at 220 nm. Solutes: (1) oxamyl; (2) methomyl; (3) de-isopropylatrazine; (4) monocrotophos; (5) fenuron; (6) metatritron; (7) de-ethylatrazine; (8) chloridazon; (9) carbendazim; (10) aldicarb; (11) aminocarb; (12) metribuzin.

However, this sorbent is not selective so many compounds are co-extracted, especially humic and fulvic substances - as the samples had been previously acidified.

Carbonaceous sorbents. Carbonaceous sorbents were first reported to be packed in on-line precolumns by Werkhoven-Goewie et al. [45,46], using pyrocarbon modified silicas and pyromodified carbon black. As with HSA/SDB precolumns, the on-

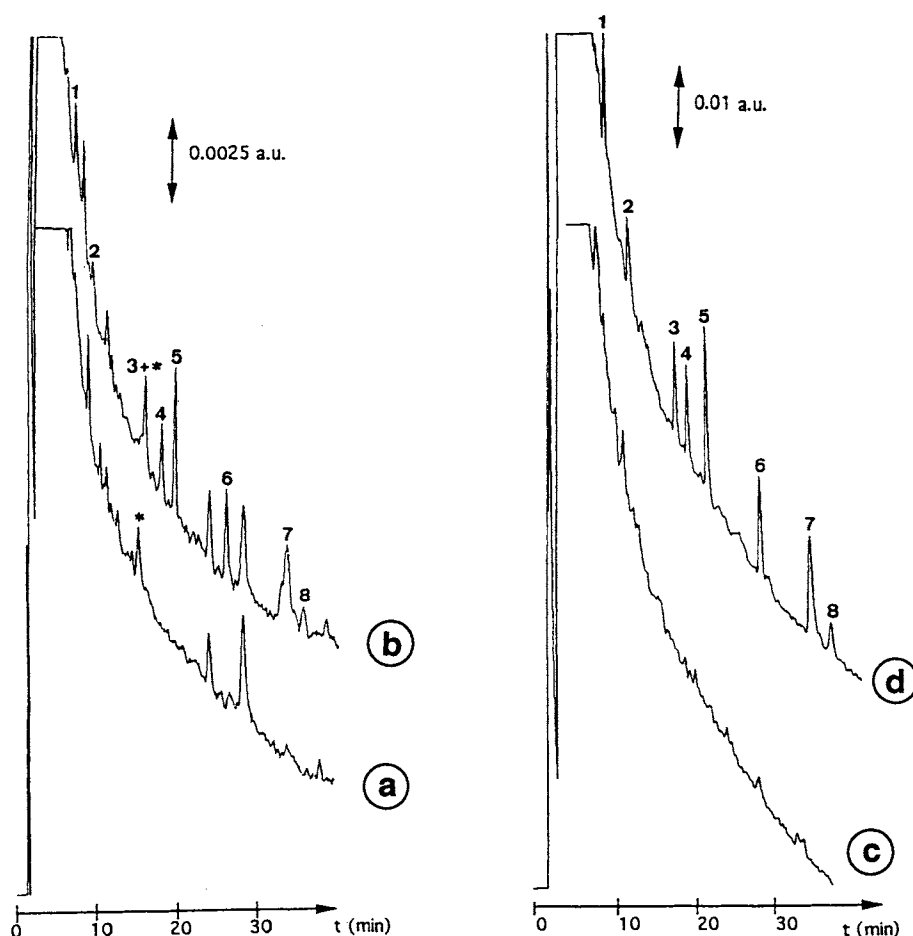


Fig. 5.7. On-line chromatograms obtained with the on-line coupling of a HSA/SDB precolumn and a PGC Hypercarb analytical column. Preconcentration of 100 ml of (a) a non-spiked drinking water sample, and (b) spiked with $0.2 \mu\text{g/l}$ of each analyte, of (c) a non-spiked river Seine water sample, and (d) spiked with $1 \mu\text{g/l}$. Samples acidified to pH 2 with perchloric acid. From Ref. [43]. Hypercarb column from Shandon, $10 \times 0.46 \text{ cm i.d.}$, acetonitrile gradient with a $5 \times 10^{-3} \text{ M}$ phosphate buffer at pH 7, from 10 to 15% acetonitrile from 0 to 5 min and up to 40% at 40 min. Flow rate, 1 ml/min, UV detection at 220 nm. Analytes: (1) clopyralid; (2) oxamyl; (3) dicamba; (4) monocrotophos; (5) picloram; (6) bentazone; (7) de-isopropylatrazine; (8) fenuron.

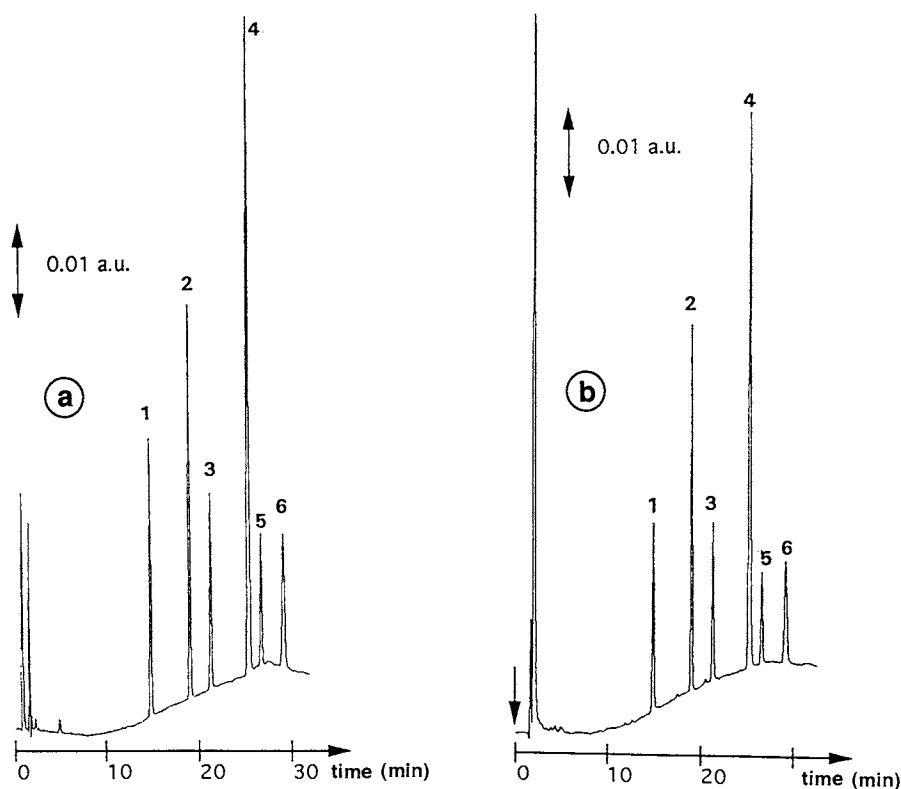


Fig. 5.8. Comparison between (a) direct injection on a PGC analytical column and (b) on-line coupling of a PGC precolumn/PGC analytical column. From Ref. [44] with permission. Water sample of 25 ml spiked with $6\text{ }\mu\text{g/l}$ of each compound; Hypercarb column from Shandon, $10 \times 0.46\text{ cm i.d.}$, acetonitrile gradient with a $5 \times 10^{-3}\text{ M}$ phosphate buffer at pH 7, 10% acetonitrile from 0 to 5 min, 15% at 10 min, 55% at 25 min. Flow rate, 1 ml/min, UV detection at 220 nm. Analytes: (1) oxamyl; (2) methomyl; (3) monocrotophos; (4) fenuron; (5) de-ethylatrazine; (6) aminocarb.

line coupling of PGC precolumns with C_{18} silica analytical columns is impossible because the polar analytes are too strongly retained by the PGC [47–50]. This is clearly shown in Fig. 5.6c, where only three compounds of the 13 were (badly) desorbed. On-line determination of hydroxychloroanilines, aminophenols and cyanuric acid was reported using both a PGC precolumn and a PGC analytical column [48]. Figure 5.8 shows clearly that when both precolumn and column have the same nature, the on-line coupling is excellent and no difference is detected between the chromatogram obtained by direct injection and by on-line preconcentration. [44].

5.2.1.3.2. Selective sorbents

Ion-exchangers. Ion-exchangers should be appropriate sorbents for selectively trapping ionic pesticides. Cation-exchangers have been used for the on-line precon-

centration of aniline derivatives [51,52]. However, it was shown that when a sample is percolated through cation-exchanger sorbents and one is looking for less than $1\text{ }\mu\text{g/l}$ of an organic cation, the much higher amounts of inorganic cations which are present in natural waters rapidly overload the cation-exchanger capacity. A chemical clean-up pretreatment consisting of oxalate precipitation of calcium ions, then EDTA complexation of metal ions, has been described for removing most of the inorganic cations before preconcentration [51,52]. Nevertheless, overloading still occurs rapidly, as demonstrated by the preconcentration study on aminotriazole [53]. When this analyte was dissolved in deionized LC-grade waters, the breakthrough was measured as 150 ml, using a $1 \times 0.2\text{ cm}$ i.d. precolumn packed with a polymer-based cation-exchanger. It was below 30 ml with drinking water samples, after the chemical pretreatment described above. In other work, a low volume, 10 ml, was percolated through an ion-pair-exchanger sorbent for the determination of aniline and chloridazon, without chemical pretreatment for the removal of anionic inorganic compounds [54].

For compounds that are ionizable in the pH range 2–10, direct percolation of samples through ion-exchanger sorbents can be avoided by using a two-trap system as described below.

Metal-loaded sorbent. Pesticides that can form complexes with metal ions can be preconcentrated selectively by metal-loaded sorbents. A silica containing the functional group 2-amino-1-cyclopentene-1-dithiocarboxylic acid (ACTA) loaded with platinum(IV) has been shown to retain anilines irreversibly from water [55]. Thus, interfering aniline could be removed in the determination of phenylurea herbicides. An Ag(I)-oxine sorbent has also been used for a rapid determination of buturon in water [56].

In recent years, there has been no further development of such sorbents, almost certainly because they are too specific and not suitable for multiresidue analysis.

Immunosorbents. The development of highly selective sorbents involving antigen-antibody interactions has occurred mainly for the handling of biological samples. Two examples have recently been reported for the determination of carbofuran [57] and atrazine [58]. In these studies, the immunosorbents were not used in direct connection to the C_{18} analytical column. After percolation of samples through the immunosorbent, desorption of analytes was effected with a buffered solution and they were re-focused on a second C_{18} precolumn and then determined on-line. Another type of silica-based immunosorbent was recently studied for the on-line preconcentration of several phenylurea herbicides [59,60]. The immunosorbent precolumn was used like a simple C_{18} precolumn, with direct coupling and desorption with a water-acetonitrile gradient. Figure 5.9 shows the selectivity that can be obtained with such a sorbent, especially for surface water. After 10 min, the baseline corresponding to the analysis of a 50 ml sample of river Seine water is as clean as that from 50 ml of drinking water. Eleven phenylureas of the 13 used for spiking the samples are recovered, thus showing the potential of the immunosorbent for screening compounds of

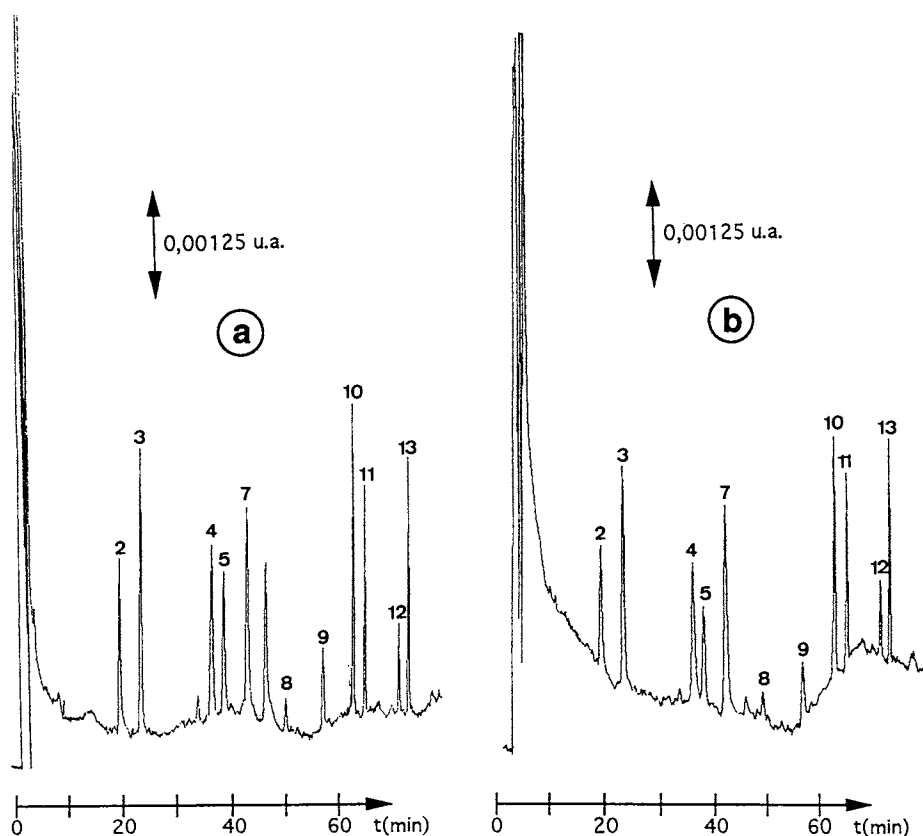


Fig. 5.9. Immuno-preconcentration of 50 ml of (a) drinking water sample and (b) river Seine water each spiked with $0.5 \mu\text{g/l}$ of a mixture containing thirteen phenylureas. Precolumn, $3 \times 0.46 \text{ cm i.d.}$ packed with a silica-based sorbent with immobilized anti-chlortoluron antibodies; analytical column, Supelcosil LC-18-DB, $25 \text{ cm} \times 0.46 \text{ cm i.d.}$; acetonitrile–water gradient; UV detection at 244 nm. Peak numbers: (2) metoxuron, (3) monuron, (4) methabenzthiazuron, (5) chlorotoluron, (7) isoproturon, (8) difenoxuron, (9) buturon, (10) linuron, (11) chlorbromuron, (12) diflubenzuron, (13) neburon. From Ref. [60].

the phenylurea class. Only fenuron and fluometuron were not recovered. The immuno-precolum was easily regenerated and re-used on-line more than 50 times without damage. Further development of immunosorbents is expected, owing to their high selectivity and their ability to trap compounds independently of their polarity or high water-solubility.

5.2.1.4. Precolumns in series

The on-line configuration usually incorporates a single precolumn. However there are many examples where more selectivity can be obtained by using two precolumns.

5.2.1.4.1. Fractionation. When the sample matrix is very complex, a pre-fractionation into polarity groups (non-polar, moderately polar, and cationic analytes) can be performed using three precolumns in series packed respectively with C_{18} silica, apolar copolymer, and a cation exchanger. This has been applied to the control of several pollutants in waste waters [52]. Each precolumn was separately eluted on-line. Brouwer et al. [54] have developed a system for the rapid determination of pesticides and degradation products, including aniline, chloroaniline and ionizable analytes. The system contained two precolumns in series, both packed with the apolar copolymer PLRP-S. The second precolumn was loaded with sodium dodecylsulfate before use, and served to trap ionic compounds. With 10 ml water samples, adjusted to pH 3, the fractionation between neutral and acidic compounds was performed allowing detection in the low $\mu\text{g/l}$ range in tap water.

5.2.1.4.2. Interference removal. Fractionation can also be performed in order to have a part of the interfering compounds on the first precolumn and the analyte of interest on the second one. The coupling of two precolumns, packed with C_{18} silica and with PRP-1 respectively, was studied for the preconcentration of phenylureas [61]. On the first C_{18} precolumn, the breakthrough values of the analytes were estimated to be 50 ml. By increasing the sample volume to 500 ml, about 80–90% were therefore trapped on PRP-1. When surface-waters containing large amounts of organic material were handled, this fractionation was shown to be useful for quantitative determination below $0.5 \mu\text{g/l}$ [52]. The first C_{18} precolumn acts as an interferent-filter for many apolar compounds, whereas the second traps the analyte of interest with a lower background in the baseline.

5.2.1.4.3. Apolar and ion-exchanger sorbents in series. As pointed out above, natural water samples cannot be percolated directly through ion-exchanger sorbents, because the chemical pretreatment by precipitation with oxalate and complexation with EDTA is inadequate for removing inorganic cations which thus prevent the percolation of sample volumes above 50 ml. For ionizable analytes in the pH range 1–13, a two-step preconcentration first proposed by Nielen et al. [62] avoids the direct percolation of samples through the ion-exchanger sorbents. It is based on the fact that solutes are retained on the PRP-1 sorbent when in their neutral form but not in their ionic form. This approach was applied to chlorotriazines and their hydroxylated derivatives which have ionization constants around 2 and 5, respectively [32,63]. The water sample, adjusted to pH 7, was percolated through a single PRP-1 precolumn. Then, this precolumn was coupled to a second one packed with the cation-exchanger precolumns and a small volume of well deionized water containing 25% acetonitrile, adjusted to pH 1, allowed the triazine herbicides to be desorbed from the PRP-1 precolumn, transferred and re-concentrated on the cation-exchanger precolumns. This second precolumn was then eluted on-line. Detection limits below $0.1 \mu\text{g/l}$ could easily be obtained in surface water samples. Although no breakthrough had

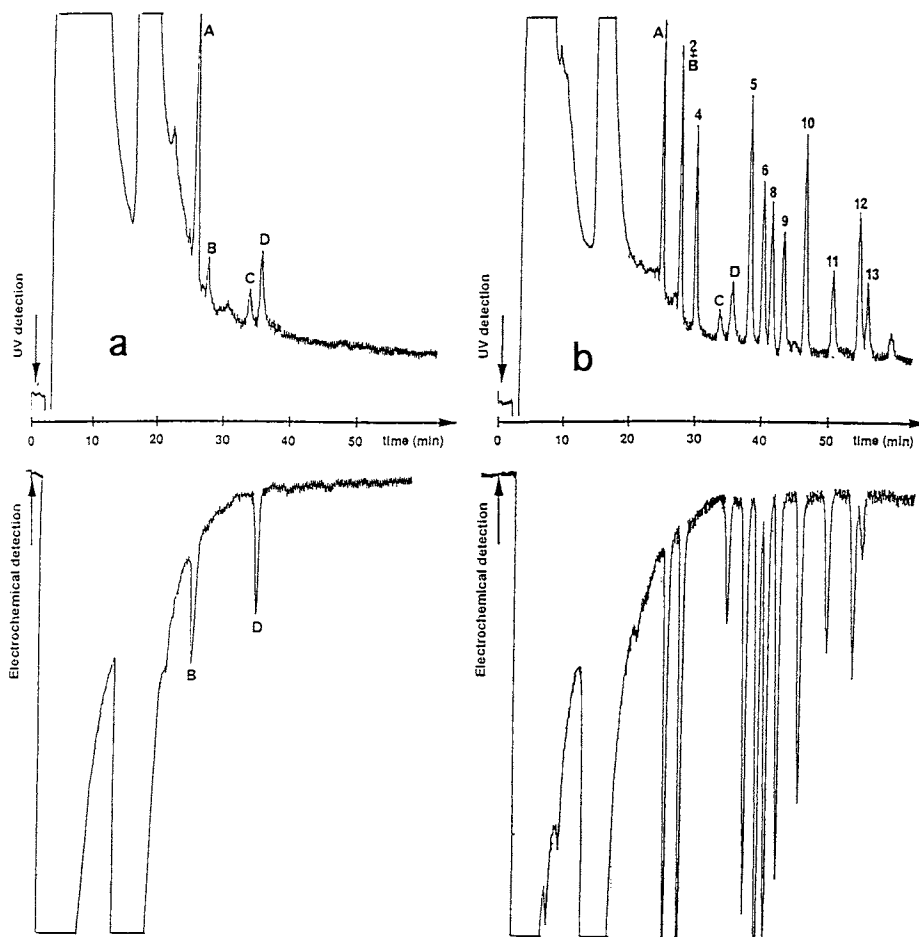


Fig. 5.10. Preconcentration and on-line analysis of a 500 ml sample of river Seine water (a) non-spiked and (b) spiked with $0.5 \mu\text{g/l}$ of each aniline derivative. Peak numbers: (2–4) monochloroanilines; (5–9) chloro-methylanilines; (9–13) dichloroanilines. Preconcentration by percolation of samples through a $15 \times 3.2 \text{ mm}$ i.d. precolumn packed with PRP-1; then transfer to a second precolumn, $10 \times 2 \text{ mm}$ i.d. packed with a cation exchanger with 3 ml of de-ionized water adjusted to pH 1 with perchloric acid containing 25% of acetonitrile. On-line desorption of the cation-exchanger content onto a C_{18} analytical column ($25 \times 0.46 \text{ cm}$ i.d.) eluted by an acetonitrile-water gradient buffered at pH 5. UV detection at 245 nm, 0.02 a.u.f.s. Electrochemical detection at 0.95 V (vs Ag/AgCl), $5 \mu\text{A}$ f.s. From Ref. [64] with permission.

occurred on the PRP-1 precolumn, the single PRP-1 precolumn could not provide these low detection limits because of numerous interferences. The selectivity provided by the cation-exchanger is illustrated in Fig. 5.10 which shows the on-line chromatogram corresponding to river Seine water spiked with $0.5 \mu\text{g/l}$ of some chloroaniline derivatives after the two-step preconcentration [64].

One advantage of the two-step preconcentration just described is that the size of the PRP-1 precolumn can be increased in order to trap more polar analytes [32,65]. The breakthrough volumes of de-isopropylatrazine, de-ethylatrazine, and hydroxyatrazine are below 100 ml on a conventional 10 mm \times 0.2 mm precolumn. By increasing the size of the PRP-1 precolumn to 5 cm \times 0.6 cm i.d. it was possible to detect less than 50 ng/l of these three rather polar degradation products in river Seine water samples as shown in Fig. 5.11.

Anion-exchangers have also been combined with PRP-1 for a two-step preconcentration. Applications to phenol and phenoxyacid herbicides have been presented [62,66,67].

Polymer-based ion-exchangers are preferred to silica-based sorbents because of their larger pH range of application and their higher capacities.

5.2.1.5. *Quantitative analyses, quality assurance and validation*

Good accuracy is a prerequisite for water analyses which are currently performed for quality control of water for human consumption, and of environmental contamination levels. In the framework of the BCR programme (now Standards, Measurement and Testing Programme of the European Commission), emphasis has been put on interlaboratory studies and experience has been gained on the main sources of errors in trace analysis [68,69]. One important source lies in the sample pretreatment, and methods that can eliminate the risks of contamination and losses should be employed. A second important source of errors is in the calibrations and peak-overlaps. The high sample throughput of on-line SPE-LC is certainly the major reason for its growing use in routine analyses and it removes the first source of errors. On-line techniques are also best suited for this, and reproducibility of results has been obtained. Quantitation methods and accuracy have received less attention.

5.2.1.5.1. Reproducibility. One advantage of automation in on-line preconcentration is that more reproducible results can be expected, provided the precolumns are packed with the same amount of sorbent and have the same efficiency. The overall reproducibility of the method includes both the reproducibility of the preconcentration and of the LC system.

The repeatability of peak-areas and heights obtained by direct-loop injections into the analytical column has been studied, using an acetonitrile gradient for the analytical separation. The relative standard deviation (RSD) was between 3 and 7%, and 3 and 5% for measurements of peak areas and peak heights, respectively [24]. In the same study, the reproducibility between cartridges was measured by preconcentrating 50 ml of LC-grade water spiked with 0.5 μ g/l of pesticides, using a Prospekt system with a new precolumn packed with the PLRP-S copolymer in each run. The RSD was around 10% ($n = 5$) for measurements of both peak areas and peak heights. RSD values below 10% have been also confirmed in other studies [25,70], thus indicating that the precolumns were packed under reproducible conditions. The flow rate

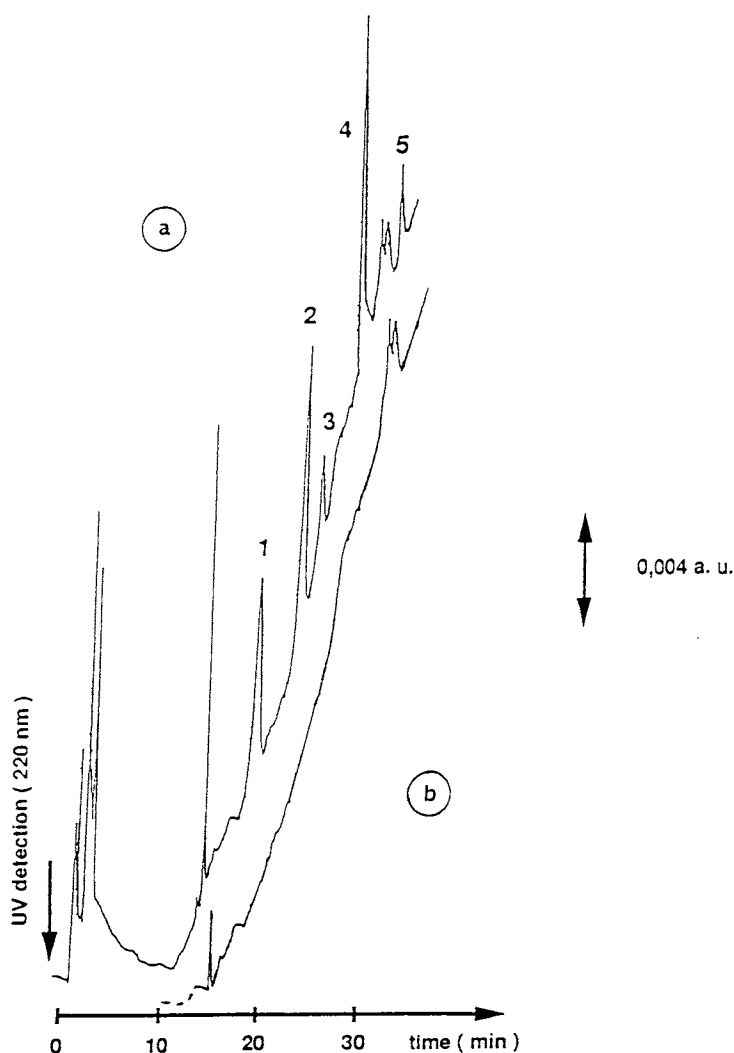
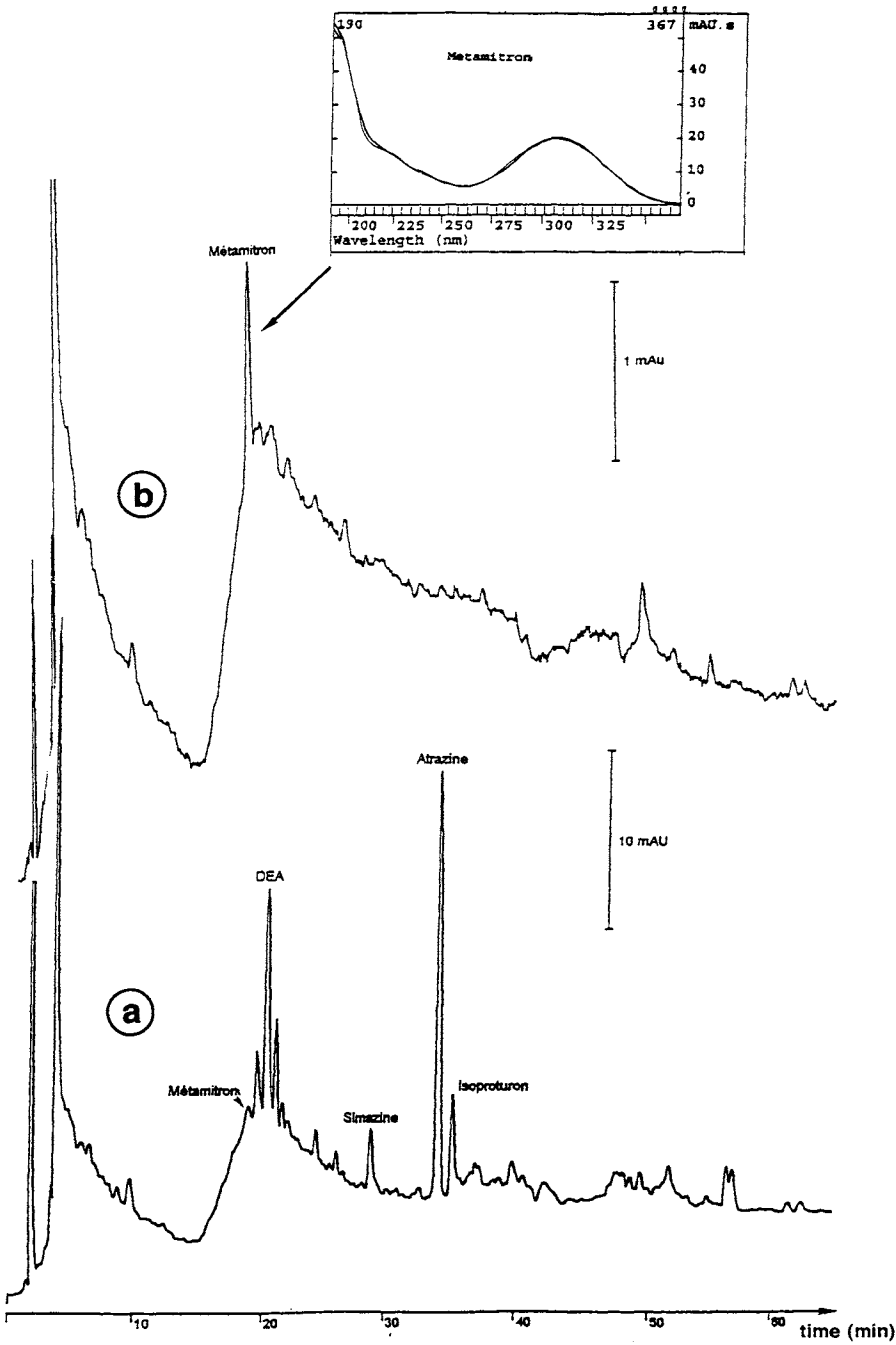


Fig. 5.11. Two-step on-line analysis of a 500 ml river Seine water sample, (a) non-spiked and (b) spiked with 50 ng/l of each compound. Peak number: (1) de-isopropylatrazine, (2) de-ethylatrazine, (3) hydroxyatrazine, (4) simazine, (5) atrazine, Preconcentration using a 5×0.6 cm i.d. precolumn and transfer to the cation-exchanger precolumn (1×0.2 cm i.d.) with 4 ml of perchloric acid solution at pH 1 containing 35% of acetonitrile. On-line elution of the cation-exchanger to a 25×0.46 cm i.d. C_{18} column with a methanol–water gradient. UV detection at 220 nm, 0.08 a.u.f.s. From Ref. [32], with permission.

applied for the preconcentration varied from 1 to 5 ml/min and the same average 10% RSD was observed [29].

In the framework of the Rhine Basin programme, an automated LC monitoring



system (SAMOS-LC, or System for Automated Monitoring of Organic Compounds in Surface Waters) has been studied extensively. The procedure includes the loading of 100–150 ml of surface water onto PLRP-S precolumns of a Prospekt device at 5 ml/min. The on-line analysis is carried out using a C₁₈ analytical column with an acetonitrile gradient at pH 3. The data are automatically evaluated, with the production of a report for compounds present at, or above, a certain concentration level between 1 and 3 µg/l [29]. The reproducibility of the retention times with a set of 25–30 pesticides was excellent with a RSD value of 0.2–1.5% ($n = 20$). At an analyte concentration of 1 µg/l, the RSD of peak areas was in the range 1–15%, with a new precolumn in each run. The highest RSD were observed only for analytes eluting between 12 and 25 min and were explained partly by matrix interferences and partly by breakthrough of the more basic compounds on the PLRP-S cartridges [22].

5.2.1.5.2. Robustness. The SAMOS system was made to act as an early warning system for use in the field. The robustness of the system was studied in two laboratories during 5- and 7-month periods. No major problem was encountered for over 1000 analyses, apart from the exchange of a deuterium lamp and clogging of the pre-concentration system with non-filtered waters [22,29].

5.2.1.5.3. Different methods of quantitation. The above studies have shown that good reproducibility is observed with on-line SPE-LC systems. The accuracy is also of prime importance and several means of quantitation can be used. With on-line systems, it is not advisable to carry out quantitative analysis by comparison with direct injections. First, the volume of many injection loops is specified to an average accuracy of 20% and calibration of a loop is a rather delicate and time-consuming operation. This does not have to be considered with off-line procedures because the same loop is used for both analysis of unknown extracts and construction of calibration curves. Secondly, slight but imperceptible band-broadening may occur.

For the above reasons, any quantitation method (calibration curves, standard addition, etc.) should be performed using the whole procedure, i.e., with the same experimental conditions (same types of precolumns, sample volume, analytical column, and on-line gradient elution) as selected for the analysis of unknown water samples. Therefore, it is not necessary to know the recovery of the extraction process for each analyte. When possible, it is better to handle a sample-volume lower than the lowest

Fig. 5.12. On-line analysis of 150 ml of a ground water sample naturally contaminated with metamitron (0.12 ± 0.02 µg/l), de-ethylatrazine, (0.61 ± 0.05 µg/l), simazine (0.05 ± 0.01 µg/l), atrazine (0.42 ± 0.03 µg/l) and isoproturon (0.48 ± 0.03 µg/l). Identification by retention time and by the match between spectra of the reference compound in a library and of the unknown peak. Quantitation using the MCA software of the DAD detector. Experimental conditions as in Fig. 5.3; chromatogram at 220 nm (a) and at 306 nm (b). In the insert, confirmation of metamitron by the UV spectrum.

breakthrough volume for more reproducible results. However, when multiresidue analyses are carried out, the sample volume is selected in order to detect most of the compounds at the required level. With a sample volume of 150 ml and using the Prospekt cartridges packed with PLRP-S, the recoveries of de-isopropyl- and de-ethyl-atrazine are not 100% (see also Table 5.3 for recoveries on PLRP-S with a sample volume of 200 ml) because breakthrough has occurred on PLRPS, but it is possible to detect these compounds with reproducible results.

The methods available for quantitation use calibration curves constructed with the on-line system, or the standard addition of known amounts to the natural samples, or the use of special software now included with most diode-array detectors.

The calibration can be made by spiking LC-grade, drinking, surface or other real-life water samples. In practice, especially in multiresidue analysis, it is easier to construct calibration curves once for all the analytes and to use them for any kind of water. It has been shown that calibration curves constructed with spiked LC-grade water samples and from spiked drinking water samples were similar [24]. Good linearity and correlation coefficients were obtained in the range 0.1–1.5 $\mu\text{g/l}$. Calibration curves have also been constructed with surface waters and good linearity was obtained in the range 0.1–7 $\mu\text{g/l}$. In surface water, the calibration curves depend on the interferences which show up in the real chromatogram and on the possibility of identifying the unknown peaks and their purity. In recent years, much attention has been given to identification and peak purity in diode-array software. The identity of a compound is indicated first by agreement with the retention times in the LC analysis with the standard and by comparison of the UV spectrum of the unknown compound and that of the standard in the Library. An automatic purity screening technique can allow one to determine possible co-elution with contaminants. Figure 5.12 shows that identification of metamitron at a concentration of $0.12 \pm 0.02 \mu\text{g/l}$ was possible from its characteristic UV spectrum, even though it appeared as a very small peak emerging from a hump caused by interferences. Four other pesticides (de-ethylatrazine, atrazine, simazine and isoproturon) were also identified in this sample of ground water.

A comparison of the different quantitation methods has been carried out [24]. Figure 5.13 shows the on-line chromatogram of a non-spiked sample of River Seine water. The concentration of atrazine was found to be $0.30 \pm 0.03 \mu\text{g/l}$ using calibration curves constructed with spiked LC-grade water or drinking-water samples. The method of standard addition was also carried out and gave a concentration of $0.28 \pm 0.03 \mu\text{g/l}$. The third method involved multicomponent analysis (MCA) of the DAD and gave a concentration of $0.26 \pm 0.03 \mu\text{g/l}$. Taking the RSD of the on-line SPE-LC system into account, these results are in good agreement. Again, the data required for identification and calculation via the DAD should be introduced using the complete procedure. This method is certainly the more rapid for determination of concentration in unknown samples. However, it is advisable to compare the results given by the DAD software with those from calibration curves for results of better accuracy.

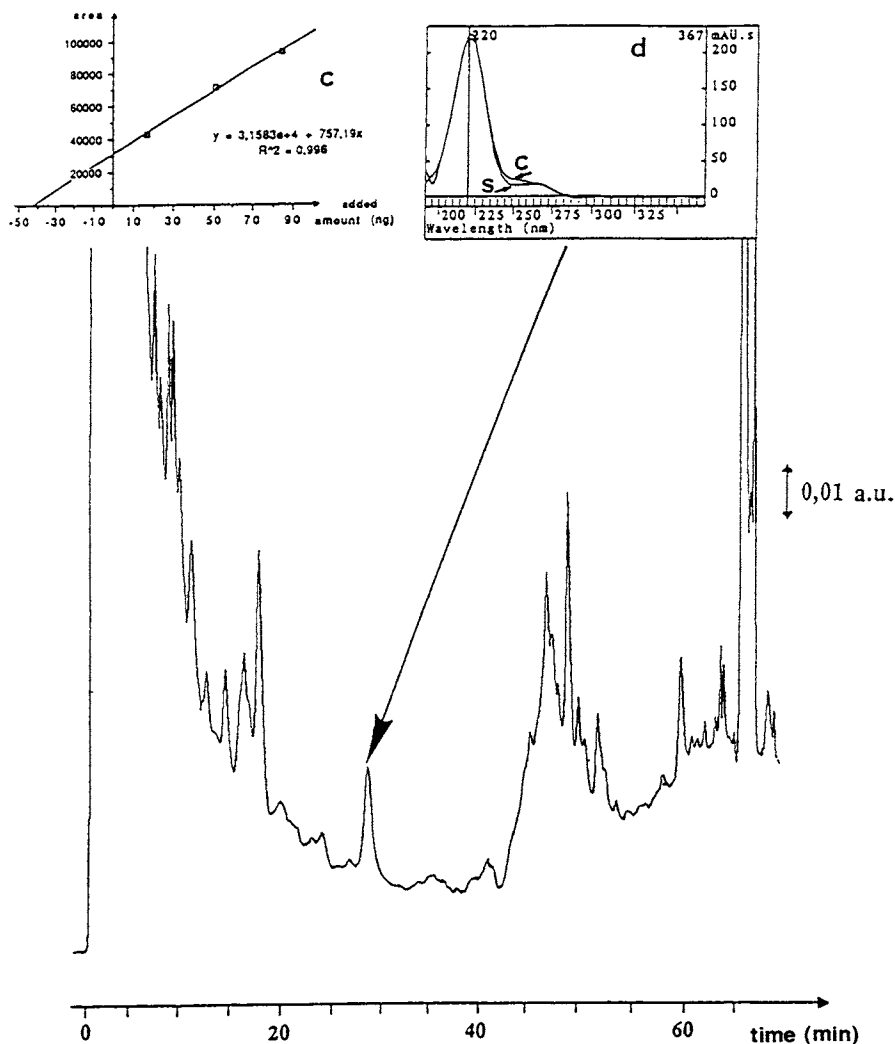


Fig. 5.13. On-line analysis a 150 ml sample of river Seine (non-spiked), and quantitation methods. On-line preconcentration on a PLRP-S cartridge (10×2 mm i.d.) using the Prospekt system. Analytical column, TSK-ODS 25×0.46 cm i.d.; mobile phase, acetonitrile gradient with 0.05 M phosphate buffer at pH 7; gradient, 30% acetonitrile from 0 to 38 min, 30–45% from 38 to 44 min, 45–47% from 44 to 52.5 min, 47–100% from 52.5 to 70 min.; detection at 220 nm. Calibration curve (c) constructed from standard addition methods (amount of atrazine added, 15, 45 and 75 ng); comparison (d) of standard spectra and unknown peak spectra of atrazine; S refers to the standard and C to the unknown compound. From Ref. [24].

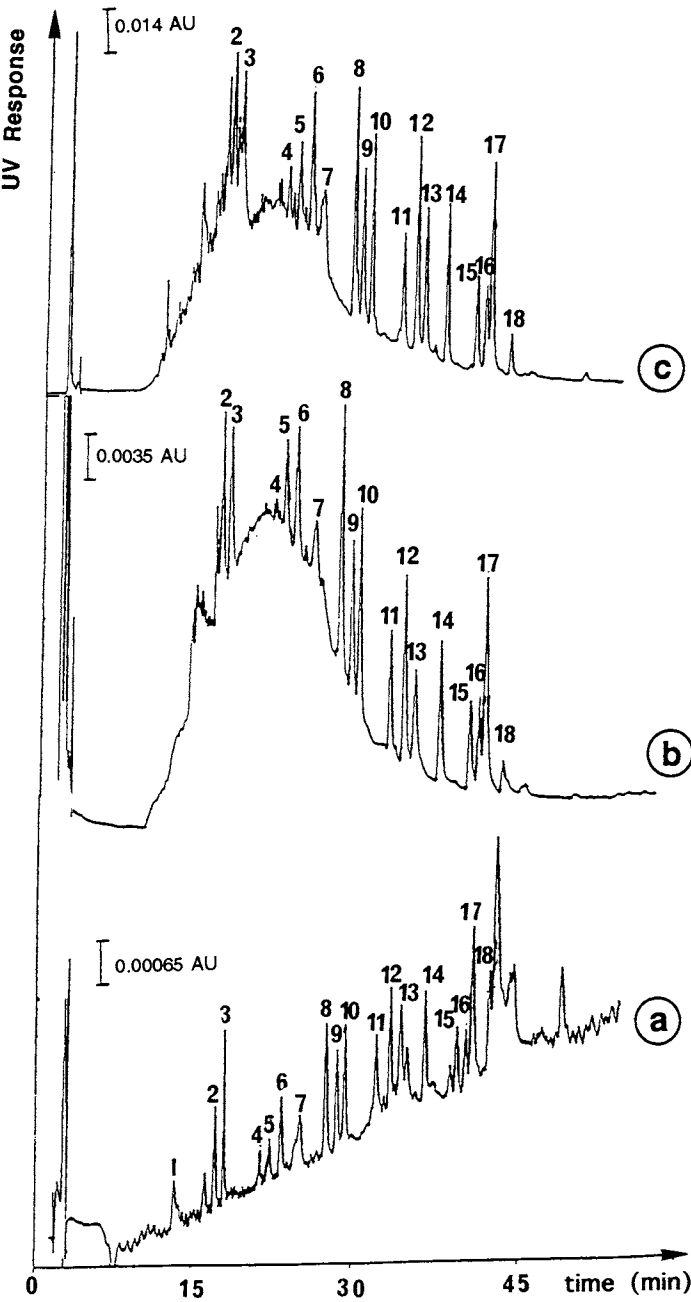
5.2.1.5.4. Validation of the on-line system. The most appropriate means for testing the accuracy of results given by an analytical procedure including a sample pre-treatment should use natural matrix reference materials which are similar to environmental ground- or surface-waters. These materials are now under study but are not yet available [71]. The actual quality control of analyses can only be performed through interlaboratory calibrations. Lacorte et al. [25,72,73] have guaranteed the validation of an automated on-line solid-phase extraction system for the first time (using a Prospekt device) by participating in the Aquacheck interlaboratory comparison study organized by the WRC (Medmenham, UK) where more conventional sample preparation methods and gas chromatographic determination were being used. The overall RSD between values obtained by the authors and the average value obtained by 14 or 15 other laboratories varied between 1.6 and 36% for atrazine and organophosphorus pesticides in finished drinking waters at levels ranging from 0.02 to 0.2 $\mu\text{g/l}$. The results reported in the interlaboratory exercise suggest that the use of the Prospekt apparatus is a robust method. This was proved in the lowest value obtained for atrazine (1.6%). The highest variations in the mean values and errors were also obtained by other laboratories and were explained by general problems encountered in the determination of organophosphorus pesticides in water samples but not the technique in use.

5.2.1.6. Application and LOD in various matrices

Various applications have been mentioned in the sections above and can be found in Ref. [10]. The suitability of the SPE-LC-DAD system for multiresidue purposes has also been pointed out. A good illustration was provided in Fig. 5.12 where metamitron was present in the ground water at a concentration level of 0.12 $\mu\text{g/l}$ and was just detected at 220 nm since this analyte was co-eluted with an interfering hump. However, the same chromatogram drawn at 306 nm illustrates the low detection limit that can be reached for this analyte and the confirmation that can be provided by the DAD when the UV spectrum presents some characteristics in the scan range. It was possible to use a full scale of 1 mV, ten times lower than that at 220 nm, because a very low background in the base line is detected at 306 nm. Unfortunately, this property does not apply to all the pesticides.

In general, the limit of determination depends primarily on the detection mode and the properties (i.e., spectral properties) of the analytes, but also on the type and

Fig. 5.14. Chromatogram of 150 ml of (a) LC-grade water spiked with 0.1 $\mu\text{g/l}$ of a mixture of pesticides, (b) drinking water spiked with 1 $\mu\text{g/l}$ of the same mixture and (c) River Rhine water spiked with 5 $\mu\text{g/l}$. Peak numbers: (1) carbendazim; (2) metamitron; (3) chloridazon; (4) aldicarb; (6) simazine; (7) 2-chloroaniline; (8) atrazine; (9) diuron; (10) monolinuron; (11) warfarin; (12) linuron; (13) 2,3-dichloroaniline; (14) barban; (15) dinoterb; (16) dinoseb; (17) pentachlorophenol; (18) phoxim. Precolumn: 1 cm \times 0.2 cm packed with PLRP-S; acetonitrile–water gradient, UV DAD detection at 230 nm. From Ref. [21].



matrix of waters that are analyzed. There is always a great difference (up to a factor of 10) between LODs obtained in LC-grade water and those obtained in ground or drinking waters. LODs in contaminated surface waters are also higher (about 5 times, depending on the organic carbon content) than those observed in drinking waters [21–29]. This is illustrated in Fig. 5.14, where three types of water were analyzed with the same experimental conditions and spiked at different levels [21]. The low detection limits which are measured with LC-grade waters (Fig. 5.14a) are not obtained with the drinking water samples of Fig 5.14b, because of the hump caused by humic compounds. It is impossible to record the chromatogram with the same attenuation scale of the detector. In the Rhine river water (Fig. 5.14c) the hump is larger, so the spiked level was $5\text{ }\mu\text{g/l}$. It is obvious from this example that the LOD depends on the amount of interfering compounds: these can appear as a hump in the middle of the chromatogram or as a large peak at the beginning. The LOD depends on the form of these interfering peaks, and these can be modified by the shape of the gradient applied for the separation [24]. As a general rule, when many compounds are studied and cover a large range of polarity, the gradient applied begins with a low content of acetonitrile and ends with 100% acetonitrile after about 60 min. This was the case in the screening method reported in Fig. 5.14. The LOD can be lowered by more appropriate gradients which usually allow the analysis of compounds in a more limited range of polarity.

A survey of the results for many drinking or ground waters indicates that the interferences are not too numerous. As shown by Fig. 5.3, the limit of determination of $0.1\text{ }\mu\text{g/l}$ is easily obtained for most pesticides using UV detection and with the handling of 150–300 ml of sample. The LOD is often lower, around 10–30 ng/l, which allows quantitative analyses at the $0.1\text{ }\mu\text{g/l}$ level. Only the more polar compounds and/or degradation products such as de-isopropylatrazine are difficult to determine at levels lower than $0.1\text{ }\mu\text{g/l}$.

In surface waters, LOD of $0.1\text{ }\mu\text{g/l}$ can also be obtained, as can be estimated from Fig. 5.15 where 150 ml of river Seine samples were used, spiked with $0.1\text{ }\mu\text{g/l}$ of a mixture of triazines (Fig. 5.15a) and phenylureas (Fig. 5.15b). Not all the analytes are detected because a large peak observed at the beginning of chromatogram masks detection of the more polar analytes. From our own experience, and from published works, pesticides that are easily determined at the $0.1\text{ }\mu\text{g/l}$ level in drinking or ground waters are also easily determined at the $0.5\text{ }\mu\text{g/l}$ level in (rather) contaminated surface waters. This is shown in Fig. 5.16 by the on-line analysis of a river Marne sample spiked with $0.5\text{ }\mu\text{g/l}$ of the same pesticides as the drinking water sample in Fig. 5.3. The chromatograms are very similar, the experimental conditions being the same apart from the attenuation range of the UV detector which is four times higher in Fig. 5.16. However, for many pesticides, the LOD are far below the $0.5\text{ }\mu\text{g/l}$ range, as can be seen from the height of some peaks at 220 nm.

It must be pointed out that the LODs discussed above were obtained in surface waters without any clean-up. LODs in the low $\mu\text{g/l}$ range are sufficient for early

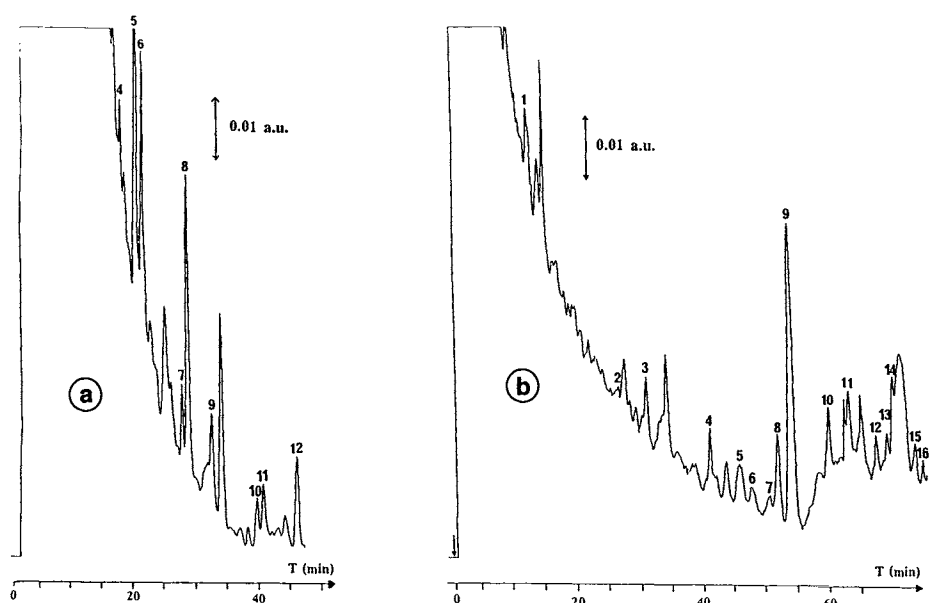


Fig. 5.15. On-line analysis of 150 ml of surface water (Seine) spiked with (a) $0.1 \mu\text{g/l}$ of 12 triazines, and (b) $0.1 \mu\text{g/l}$ of 16 phenylureas. Triazine peak number: (4) hexazinone, (5) simazine, (6) cyanazine, (7) simetryn, (8) atrazine, (9) prometon, (10) sebutylazine, (11) propazine, (12) terbuthylazine. Phenylurea peak numbers: (1) fenuron, (2) metoxuron, (3) monuron, (4) methabenzthiazuron, (5) chlorotoluron, (6) fluometuron, (7) monolinuron, (8) isoproturon, (9) diuron, (10) difenoxuron, (11) buturon, (12) linuron, (13) chlorbromuron, (14) chloroxuron, (15) diflubenzuron, (16) neburon. Precolumn: PLRP-S cartridge (1 cm \times 2 mm i.d.) from the Prospekt; analytical column, Varian ODS 25 \times 0.46 cm i.d. From Ref. [24].

warning and monitoring purposes. By changing the conditions, lower LODs can be obtained, thus allowing studies of the environmental behaviour of a restricted number of pesticides.

For the more polar range of compounds, the on-line system PGC/PGC could provide accurate determination of the analytes. In a long-term survey of a ground water source, monitoring using the PLRP-S precolumn and C_{18} analytical column on-line system indicated constant and rather high amounts of atrazine and de-ethylatrazine, with average concentrations of 0.5 and $0.6 \mu\text{g/l}$, respectively. A representative chromatogram is shown in Fig. 5.12 [44,74]. Because of the bad detection obtained with this system for the second metabolite de-isopropylatrazine, a PGC precolumn-PGC analytical column coupling was used. Figure 5.17 shows the advantage of such a system, since de-isopropylatrazine (DIA) is eluted after de-ethylatrazine (DEA) and can easily be delayed to 40 min in the chromatogram, after the interfering compounds. The breakthrough volume of DIA on PGC is above 100 ml so that detection limits using 100 ml samples are in the low- $0.1 \mu\text{g/l}$ range in LC-grade water, as

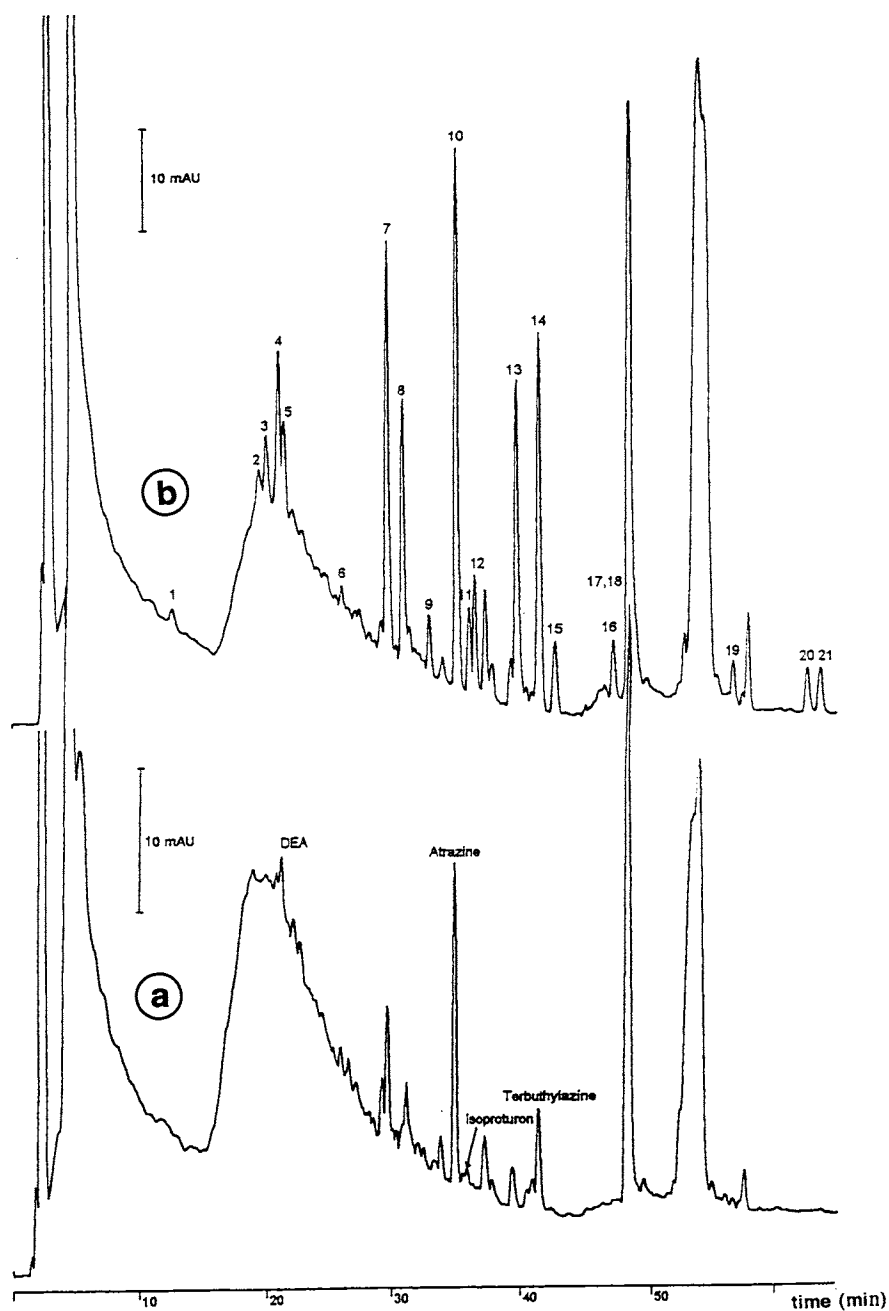


Fig. 5.16. On-line analysis of, (a) 150 ml of non-spiked river Marne water, and (b) 150 ml of the same sample spiked with 0.5 $\mu\text{g/l}$ of a mixture of 21 pesticides. Experimental conditions and peak identities as in Fig. 5.3.

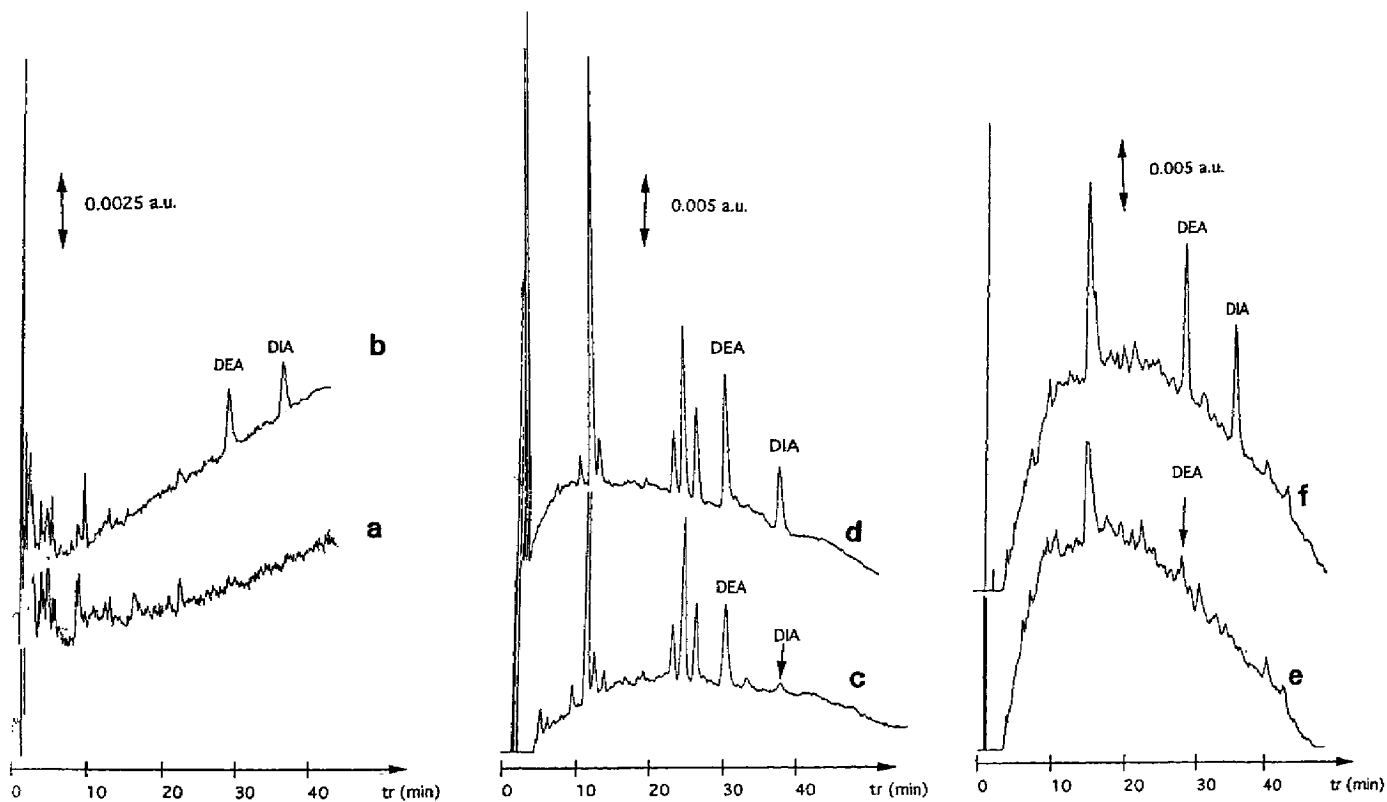


Fig. 5.17. On-line preconcentration of different aqueous matrices using the on-line coupling of a PGC precolumn and PGC analytical column. Samples: (a) 100 ml of non-spiked LC-grade water; (b) spiked with $0.2 \mu\text{g/l}$ of DEA and DIA; (c) 100 ml of non-spiked ground water; (d) spiked with $0.5 \mu\text{g/l}$ of DEA and DIA; (e) 100 ml of non-spiked Seine river water, and (f) spiked with $0.5 \mu\text{g/l}$ of DEA and DIA. Hypercarb column from Shandon, $10 \times 0.46 \text{ cm i.d.}$, acetonitrile gradient with a $5 \times 10^{-3} \text{ M}$ phosphate buffer at pH 7, 15–35% acetonitrile from 0 to 40 min. Flow rate, 1 ml/min, UV detection at 220 nm. From Ref. [44] with permission.

shown in Fig. 5.17a,b. In the non-spiked ground water (Fig. 5.17c,d), DEA was confirmed at a concentration of $0.6\text{ }\mu\text{g/l}$ and the concentration of DIA was $0.05 \pm 0.01\text{ }\mu\text{g/l}$. The system allows determination of these two metabolites in surface water with detection limits as low as the order of $0.1\text{ }\mu\text{g/l}$, since Fig. 5.17f shows nice peaks from the on-line analysis of a 100 ml river Seine sample spiked at the $0.5\text{ }\mu\text{g/l}$ level.

Most SPE-LC systems are equipped with a UV DAD mode, because this is a powerful tool for the identification of pesticides having UV spectra. However, the use of more specific detectors can improve the LOD, as was shown in Fig. 5.10 for the detection of chloroanilines using UV and electrochemical detection (ECD). With the combination of selective trace enrichment and the sensitive ECD, it was possible to determine chloroanilines with LOD lower than 30 ng/l in 150 ml of river water. Among the pesticides, phenylureas are easily detected by the electrochemical detector [61,75,76]. There are also a few pesticides that are naturally fluorescent and can be thus detected [26]. However, in the same study [26], it was reported that some phenoxyacid herbicides exhibit quenching interferences from the matrix of estuarine water samples.

The clean-up procedure that can be involved in the technique can be merely a washing of the SPE cartridge with a few millilitres of a mixture of water and an organic solvent that would elute the more polar analytes. However, many analyses are for multiresidue purposes with polar and apolar pesticides to be determined at the same time, and clean-up is then almost impossible.

In conclusion, the on-line SPE-LC system is robust and reliable for the trace analysis of pesticides in environmental waters. It can be fully automated and works as an early warning or on-site monitoring system. It can also be used as a powerful routine tool in the laboratory. The numerous applications have shown this method to be well suited for multiresidue analysis.

5.2.1.7. New trends: rapid screening with a single short column for trace enrichment and separation

Recently, an interesting method was derived from the on-line SPE-LC system for the rapid screening of water samples for polar organic pollutants. In this method, a single short column is used to accomplish sample extraction, trace enrichment and separation [77,78]. The screening of water samples is performed with substantially simplified conditions and at reduced cost compared with the arrangement shown in Fig. 5.1. Figure 5.18a shows the analysis of a surface water sample spiked with $5\text{ }\mu\text{g/l}$ each of atrazine and metazachlor, using a $20 \times 4.6\text{ mm}$ i.d. short column packed with $5\text{ }\mu\text{m}$ C_{18} silica. In the first step (T1) the column is purged with 10 ml of acetonitrile, and in T2 with 10 ml of water; in the third step (T3) 10 ml of sample is loaded onto the short column. Then the gradient is started and the separation performed. Since the system is simplified as far as possible, there is no switch-to-waste during sampling and the soluble matrix is seen as a large peak. This method is made

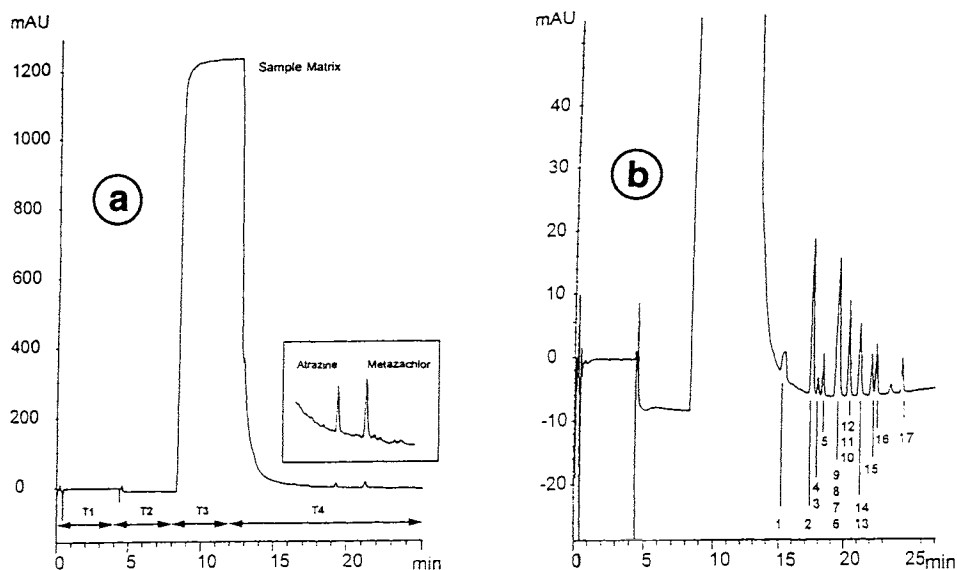


Fig. 5.18. (a) River Rhine water sample spiked with $5 \mu\text{g/l}$ atrazine and metazachlor and (b) tap water spiked with $5 \mu\text{g/l}$ of a mixture of 17 pesticides using an automated on-line system with a $20 \times 4.0 \text{ mm}$ i.d. short column used for both enrichment and separation. From Ref. [77], with permission. Steps: (a) T1, purge with acetonitrile; T2, wash with 10 ml of water; T3, load 10 ml of sample; and T4, gradient elution. (b) Gradient 2–92% acetonitrile in 30 min. Solutes: (1) de-ethylatrazine; (2) metoxuron; (3) hexazinone; (4) simazine; (5) cyanazine; (6) methabenzthiazuron; (7) chlortoluron; (8) atrazine; (9) monolinuron; (10) diuron; (11) isoproturon; (12) metobromuron; (13) metazachlor; (14) sebutylazine; (15) terbutylazine; (16) linuron; (17) metolachlor.

for environmental screening where, in most instances, only one or a few compounds of interest will occur at a certain point in time. Therefore, an analytical column is no longer required to offer an analytical window within which all the analytes of interest are separated from each other, but only a window within which they elute as a group with more or less resolution. The only required feature is a sufficient discrimination of the sample matrix from the analytes and sufficient capacity to give high recovery and retention of analytes during sampling. This is illustrated in Fig. 5.18b where the same short column as in Fig. 5.18a is used and where ten peaks are observed for 10 ml of tap-water spiked with a mixture containing seventeen pesticides. The most polar compound in the mixture is de-ethylatrazine, which is separated from the sample matrix but gives a broad peak resulting from the enrichment.

5.2.2. On-line solid-phase extraction and liquid chromatography with post-column reaction detection

Another means of improving selectivity and sensitivity uses post-column derivati-

zation which consists of converting the compounds, after their separation, into products with better detection characteristics. Post-column reaction is usually carried out for compounds with poor UV characteristics. As increases in both sensitivity and selectivity are sought, fluorescence or electrochemical detection are usually preferred over UV. Obviously, the conversion into fluorescent compounds is more popular. The well-known advantages of post-column detection are: (i) the analytes are separated in their original form, which often permits the adoption of published separation procedures; (ii) the formation of artefacts plays a minor role; (iii) the reaction should be fast but does not need to be complete, and the reaction products need not be stable, since the only requirement is reproducibility; (iv) it can be carried out on-line and is easily automated. However, the reaction requires the addition of reagents via additional pumps, and band broadening in the reactor has to be avoided. The shape of the reactor used depends on whether the reaction is homogeneous or heterogeneous. The latter is more interesting, as are photoconversion procedures, because it minimizes the number of additional pumps and therefore the cost of the methods.

5.2.2.1. *On-line post-column derivatization of N-methylcarbamates*

This is certainly the most relevant example of automated post-column reaction detection in pesticides analysis. The *N*-methylcarbamates have poor UV spectral properties. Moreover, some of them are very polar, so that breakthrough occurs rapidly on the PLRP-S cartridges. As an example, the breakthrough volumes of oxamyl and methomyl are below 20 ml, using a 10 × 2 mm i.d. PLRP-S precolumn (see Table 5. 2). The post-column reaction is based on a hydrolysis by sodium hydroxide and the further addition of a solution containing *o*-phthalaldehyde and 2-mercaptoethanol (OPA/MERC) in order to convert quantitatively the methylamine formed into the highly fluorescent 1-hydroxyethylthio-2-methylisoindole [79]. The procedure is also described in the EPA Method 531.1 for *N*-methyl and *o*-(methylcarbamoyl)oxime carbamate.

In order to reduce the band broadening resulting from the overall post-column reaction the hydrolysis step was performed in a solid reactor containing an anion-exchanger or magnesium oxide [80]. Twenty parent *N*-methyl carbamate pesticides and 12 major metabolites were studied. They were first preconcentrated using an off-line cartridge packed with 500 mg of C₁₈ silica (Bond-Elut C₁₈OH from Analytichem) and recoveries were above 80% for all the analytes (except the two sulfoxide metabolites of ethiofencarb and thiofanox) with a 50 ml water sample. After elution of the preconcentrated analytes with acetonitrile, evaporation to a dry extract, and reconstitution in 1 ml of water, 100 µl samples were injected into the LC system and the carbamates were separated with a water-methanol-acetonitrile gradient. Detection was performed via post-column hydrolysis on a solid-phase (anion-exchange) catalyst and derivatization of the methylamine formed with OPA/MERC solution and fluorescence detection. The detection limits for surface waters were between 20 and 30 ng/l.

The method was modified by Chiron et al. [36] using an on-line preconcentration step with ten layers of C₁₈ Empore disk contained in a membrane holder. From 10 ml of water sample, the percent recoveries were between 70 and 100% for 15 selected *N*-methyl carbamates and transformation products. For the post-column reaction detection, the workers used the PCX 5000 carbamate post-column analysis module from Pickering Laboratories (Mountains, USA). The detection limits that could be obtained were 10 ng/l from only 10 ml of river water. This result shows the enhancement of the fluorescence detection over UV detection. Hiemstra and de Kok also modified their previous method by using an automated on-line preconcentration via the Prospekt or the OSP-2 devices, and the same post-column reaction [81]. Figure 5.19 shows the on-line analysis of a 5 ml surface water sample spiked with 19 *N*-methyl carbamates or degradation products at a concentration of 0.1 µg/l. The authors used a larger C₁₈OH precolumn than used for the experiment in Table 5.2 in order to have no breakthrough for the more polar carbamates and degradation products. The repeatability was excellent, with a RSD between 2 and 10%. The detection limits were in the range 30–50 ng/l, as with off-line preconcentration from 100 ml samples. The method proved to be suitable for monitoring these compounds in environmental waters. However, none of the compounds reported in Fig. 5.19 was detected in the river Rhine during a 6-month monitoring period.

An on-line preconcentration and a post-column reaction detection similar to that above has been described for the detection of glyphosate [82].

5.2.2.2. On-line solid reactors

The combination of an on-line SPE-LC system with an on-line post-column reaction detection involving the use of a solid reactor containing metallic copper was described for the analysis of some dithiocarbamate fungicides such as thiram and disulfiram. The reaction led to the formation of a complex which could be detected at 435 nm [83]. The post-column system is very simple and did not cause any band broadening.

5.2.2.3. On-line photochemical reactors

Using a photochemical reactor is the simplest approach to post-column reaction. This has been mainly applied to the determination of drugs in serum or plasma. Environmental applications deal with the determination of phenolic compounds. Classical methods based on SPE followed by LC with UV detection lack sensitivity and selectivity. Enhanced sensitivity is obtained by using electrochemical detection, but it is not enough for trace analysis. A fast and simple derivatization reaction has been performed which involved using a tetrabutylammonium counter-ion to extract ion-paired deprotonated phenols into an organic phase in which the derivatization agent (dansyl chloride) was dissolved [84]. The sensitivity was improved by the application of a post-column photochemical reaction, since the UV decomposition of dansyl derivatives of phenolic compounds in a methanol–water mixture leads to the forma-

tion of highly fluorescent dansyl hydroxides or dansyl methoxides. Detection limits below $0.1 \mu\text{g/l}$ were obtained for pentachlorophenol from a 500 ml sample of surface water, and below $0.5 \mu\text{g/l}$ from 100 ml samples for tri- and tetra-chlorophenols. It was not possible to detect the complete range of phenols owing to the early breakthrough volumes on the small precolumns. This problem was solved by using the more sensitive peroxyoxalate chemiluminescence detection of dansyl derivatives instead of fluorescence detection. This method was applied to the detection of a wide range of phenols including polychloro-, alkyl-, and nitro-phenols in river Rhine water, below the $0.5 \mu\text{g/l}$ level [85].

Multiresidue methods have been developed which use LC with post-column reaction detection for over 100 analytes from the US EPA's National Survey of Pesti-

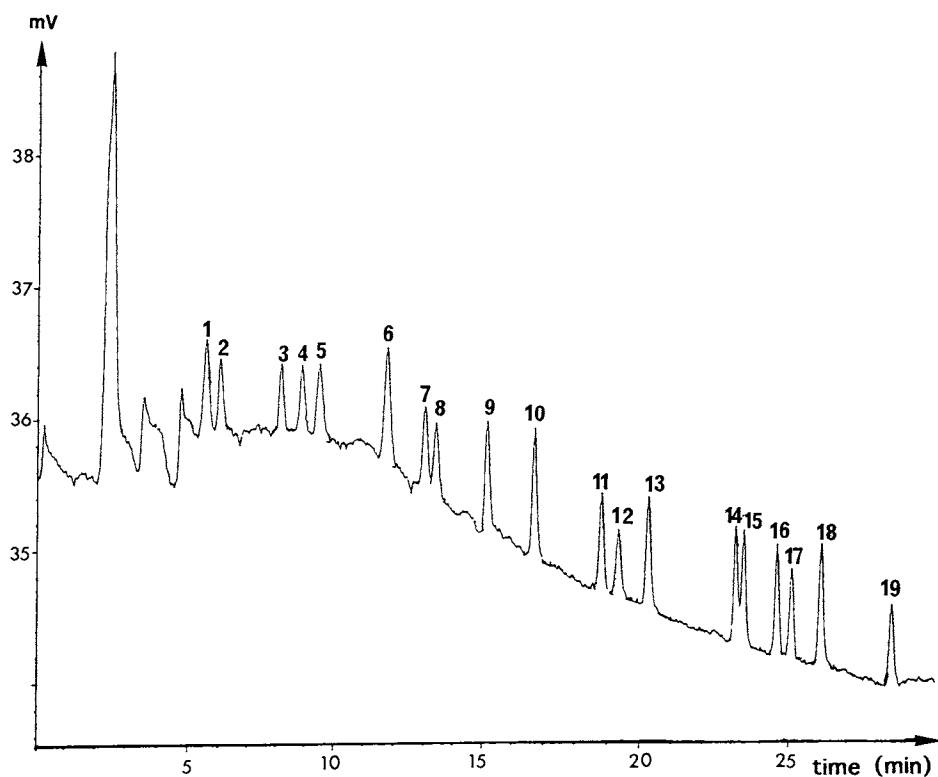


Fig. 5.19. LC chromatogram of a 5 ml surface water sample fortified with 19 *N*-methyl carbamates and metabolites at the $0.1 \mu\text{g/l}$ level after on-line enrichment on a $1 \times 0.4 \text{ cm i.d. C}_{18}\text{OH}$ precolumn using the OSP-2 system. Trimethacarb was used as an internal standard. Solutes: (1) butocarboxim SO; (2) aldicarb SO; (3) butocarboxim SO_2 ; (4) aldicarb SO_2 ; (5) oxamyl; (6) methomyl; (7) ethiofencarb SO; (8) thiofanox SO; (9) methiocarb SO; (10) thiofanox SO_2 ; (11) methiocarb SO_2 ; (12) butocarboxim; (13) aldicarb; (14) Propoxur; (15) carbofuran; (16) carbaryl; (17) ethiofencarb; (18) trimethacarb; (19) methiocarb. From Ref. [81].

cides in Drinking Water. Wells were screened for response using post-column photolysis followed by fluorescence, electrochemical, or conductivity detection [86].

In conclusion, the use of post-column reaction detection in combination with a simple on-line preconcentration allows one to enhance considerably the selectivity and the sensitivity, as shown by the low limits of determination obtained with the handling of only 5 ml sample. This is especially clear when the products are determined by using fluorescence detection. However, except for *N*-methyl carbamates, the method has been applied to a limited number of compounds and does not correspond to a multiresidue approach.

5.2.3. On-line solid-phase extraction and liquid chromatography with MS detection (SPE-LC-MS systems)

The on-line combination of LC with MS is the most powerful tool available for confirmation of the presence of pesticides and their transformation products in water matrices with no false positive determinations. Of the different LC-MS systems, thermospray (TSP) has been widely used for water analysis, usually following off-line liquid-liquid extraction (LLE) and/or solid-phase extraction (SPE) [1,87–90]. One of the assets of LC-MS systems is their suitability for analyzing a wide variety of pesticides, which makes them quite appealing for multiresidue analysis. In this respect, TSP-LC-MS has permitted the determination of various pesticides in water.

Concerning environmental regulations particle beam (PB) LC-MS was the first LC-MS technique recommended by the US Environmental Protection Agency (EPA) for the detection of pesticides in drinking water [91]. Extraction from water samples was based either on off-line LLE or SPE with the target pesticides diuron, linuron and carbaryl. More recently [92], the US EPA has applied LC-MS with the APCI interface for the determination of the same compounds and of carbamates in water following SPE. Before US EPA approval, intercomparisons should be organized with various EPA laboratories. In recent interlaboratory studies the performance of TSP and PB was compared for the determination of various pesticides and it was shown that TSP is superior to PB for the determination of carbamates (e.g., carbaryl, carbofuran [93,94] and acidic herbicides (e.g., 2,4-D and MCPA [95]) and that the limit of detection is 10–50 times lower in TSP than for PB; the former also shows a wider linear dynamic range.

In the next section we discuss the on-line SPE coupling followed by LC, using PB, TSP, electrospray (ESP), and atmospheric pressure chemical ionization (APCI) techniques for the trace analysis of pesticides in water samples. We only consider papers that have used on-line SPE followed by LC-MS and not papers dealing with LC-MS of pesticides; these were discussed in Chapter 3.

5.2.3.1. On-line SPE-LC-PB-MS systems

LC-PB-MS has been used in environmental analysis, mainly because it permits

the identification of unknown compounds. The promulgation of the EPA Method 553 that involves off-line SPE followed by LC-PB-MS has also been of help for the identification of unknowns in environmental water matrices and in solid wastes [91]. The on-line SPE with LC-PB-MS was reported by Bagheri et al. [96] and Prosen et al. [97]. This technique is used for the determination of unknown pollutants in various European river waters by preconcentrating 100–250 ml of water sample. The method permitted the determination of chlortoluron and diuron at levels varying from 50 to 250 ng/l, and the LC-PB-MS spectra matched conventional electron-impact spectra [96]. A comparison between off-line SPE and on-line SPE followed by LC-PB-MS was undertaken for triazine herbicides in water. The limits of detection (LODs) were in the range of 0.2–1.2 ppb using 200 ml of water. These LODs are relatively high compared to other LC-MS detection systems, as shown later. Using such a system, atrazine was detected at the 0.3 ppb level in a highly polluted stream from Slovenia [97]. Recently, further work was performed by Slobodnik et al. [88], involving the determination of 48 carbamates and their degradation products using 100 ml of water sample. The LODs were in the range of the ppb level for 17 out of 48 carbamates using electron impact and full scan conditions. Overall, the use of on-line LC-PB-MS is not recommended for the analysis of drinking water samples that contain pesticides since the LODs are higher, in most cases, than required by the Commission of the European Communities Drinking Water Directive of 0.1 ppb for individual pesticides.

5.2.3.2. *On-line SPE-LC-TSP-MS*

The on-line coupling of SPE and LC-TSP-MS is by far the most applied on-line SPE LC-MS system for pesticide water analysis. More than 100 pesticides have been enriched from various water types and chemical groups such as carbamates, organophosphorus compounds, phenylureas and triazines, and their corresponding metabolites from water samples [23,36,98–105]. The optimization of two on-line systems based on SPE Empore extraction disks and PRP-1 precolumns was described for the preconcentration of 30 pesticides and various transformation products included on the NPS list of the US EPA and the European Community [36], and for the characterization of phenylurea and triazine herbicides [98], respectively. As a rule, quantitation was performed by UV at 220 nm or by using a post-column reaction with fluorescence derivatization (for carbamate pesticides).

However, it is of interest to develop on-line SPE-LC-MS methods which involve the simultaneous determination of the parent pesticides and their transformation products, and to use the technique for quantitation purposes. In this respect, multiresidue methods using on-line SPE followed by LC-TSP-MS were developed for the determination of 30 pesticides of different chemical groups and for various transformation products [26,98]. They were also applied to environmental degradation studies in estuarine water of ten organophosphorus pesticides, thus facilitating the unequivocal determination of various degradation products such as 3,5,6-trichloro-2-

pyridinol, pyridafenthion-oxon, temephos-oxon, diazinon-oxon and fenamiphos sulfoxide as the main transformation products formed from chlorpyrifos, pyridafenthion, temephos, diazinon and fenamiphos, respectively. This is a useful application of the on-line SPE-LC-MS technique since it is possible to study the behaviour of pollutants in water samples at their usual application rate (20–100 µg/l) and follow the degradation during a period of 4–5 weeks, as reported [101]. In previous studies – based on off-line techniques – it was necessary to extract the pesticides and their transformation products from water samples and then to analyze the concentrated extracts. This implied a modification of the estuarine water sample submitted to degradation and, in general, it is a less sensitive method than the use of on-line SPE. Using the latter, with only 75–100 ml of sample it is possible to follow the degradation of the different pesticides during the period of study.

Figure 5.20 illustrates the analysis of a spiked river water sample determined using on-line SPE with ten C₁₈ Empore disks followed by LC-TSP-MS detection in the PI and NI mode under SIM conditions after preconcentration of 100 ml of water from Ebro estuarine river water spiked with 0.3 µg/l of a pesticide mixture. From the traces, it is clear that no matrix peak appears at the beginning of the chromatogram (20–30 min) arising from the humic substances present in the estuarine waters, which can vary according to the water-type of the river and the gradient elution performed. In this respect, it can be observed that the use of MS detection under SIM conditions “artificially” removed all kinds of interference peaks and permitted the determination of pesticide transformation products such as de-isopropylatrazine (peak 7), 3-hydroxycarbofuran (peak 8), methiocarb sulfoxide (peak 9) and de-ethylatrazine (peak 10), among others. Although all the dirty water samples were injected directly into the source of the MS apparatus, no loss of sensitivity was observed during the analysis. The LODs obtained with the method varied from 0.01 to 0.1 µg/l, depending on the compound; the values were higher for certain transformation products such as aldicarb sulfone and 3-hydroxycarbofuran and lower for atrazine, aldicarb, methiocarb and isoproturon (see Table 5.5). Although such good results are obtained using this technique – permitting one to analyze estuarine water samples with pesticides at 0.1 µg/l, without the problems associated with humic substances – problems still arise with the use of TSP, associated with difficulties in the technique of ionization. In this respect, the quantitation of compounds such as aldicarb sulfoxide, butocarboxim sulfoxide, and oxamyl was not feasible at the 0.1 µg/l level; they exhibit LODs of 0.4 µg/l. In addition, alachlor and metolachlor gave quantitation problems because they were eluted with 100% of organic modifier; with such an amount of organic modifier, large variations take place in the tip temperature of the TSP interface thus hampering their quantitation. In conclusion, although on-line-SPE-LC-TSP-MS is a very useful technique for trace analysis of pesticides in water, not all the compounds of interest can be measured simultaneously and some limitation has been noticed in the use of this technique.

Another possibility of on-line SPE-LC-TSP-MS is its use in the negative-ion

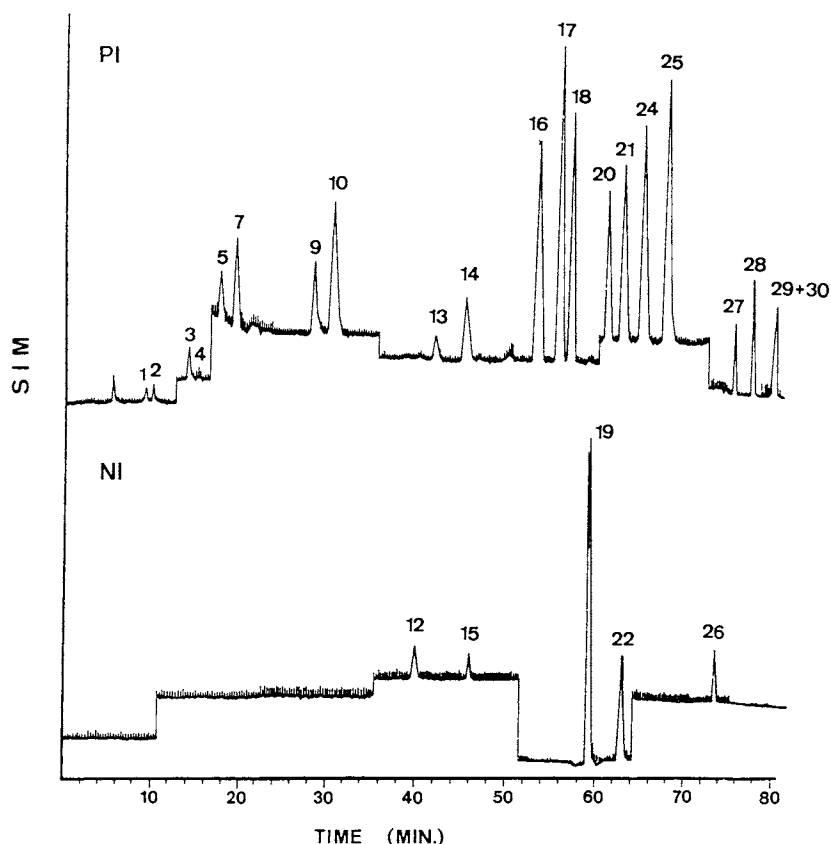


Fig. 5.20. On-line SPE LC-TSP-MS chromatogram obtained after preconcentration on C_{18} Empore disks of 100 ml of Ebro estuarine water spiked at $0.3 \mu\text{g/l}$ with (1) butocarboxim sulfoxide, (2) aldicarb sulfoxide, (3) aldicarb sulfone, (4) oxamyl, (5) methomyl, (6) 3-hydroxy-7-phenolcarbofuran, (7), de-isopropylatrazine, (8) 3-hydroxycarbofuran, (9) methiocarb sulfoxide, (10) de-ethylatrazine, (11) methiocarb sulfone, (12) 3-ketocarbofuranphenol, (13) butocarboxim, (14) aldicarb, (15) 3-ketocarbofuran, (16) simazine, (17) baygon, (18) carbofuran, (19) bentazone, (20) carbaryl, (21) chlortoluron, (22) MCPA, (23) 1-naphthol, (24) atrazine, (25) isoproturon, (26) propanil, (27) methiocarb, (28) molinate, (29 + 30) alachlor + metolachlor, obtained after preconcentrating 100 ml and 150 ml in PI and NI modes, respectively, and under SIM conditions. Precolumn packed with ten C_{18} Empore extraction disks Lichrocart cartridge column ($250 \times 4.6 \text{ mm i.d.}$) packed with $4 \mu\text{m}$ Supersphere 60 RP-8 from Merck. Gradient elution program: from 5% of A [acetonitrile–methanol–water + 0.075 M ammonium formate (40:40:20)] and 95% of B [acetonitrile–ammonium formate 0.05 M–formic acid buffer, pH 3 (10:90)] to 20% A/80% B in 15 min. From 20% A/80% B to 30% A/70% B in 20 min. From 30% A/70% B to 55% A/45% B in 20 min. Isocratic during 10 min. From 55% A/45% B to 100% A in 10 min. Isocratic during 10 min. Back to initial conditions, post run 10 min. Flow rate 0.85 ml/min.

TABLE 5.5

CALIBRATION DATA FOR SELECTED PESTICIDES (SPIKED AT 0.025, 0.1, 0.4, 0.8 AND 1.2 $\mu\text{g/l}$) AFTER PRECONCENTRATION OF 100 ml OF ESTUARINE WATER

Analyte	Calibration equation	R^2	CV (%) ^a	LOD ($\mu\text{g/l}$)
Aldicarb sulfone	$y = 69.2x + 2.56$	0.962	7	0.1
Methomyl	$y = 1.43x + 1.26$	0.975	10	0.05
De-isopropylatrazine	$y = 15.9x + 1.43$	0.915	9	0.02
3-Hydroxycarbofuran	$y = 8.31x + 1.67$	0.995	7	0.1
De-ethylatrazine	$y = 14.1x + 1.18$	0.990	8	0.02
Butocarboxim	$y = 9.21x + 1.20$	0.977	10	0.05
Aldicarb	$y = 13.5x + 1.76$	0.922	11	0.02
Simazine	$y = 18.7x + 1.45$	0.995	8	0.01
Propoxur	$y = 24.8x + 1.62$	0.931	13	0.02
Carbofuran	$y = 152.4x + 7.51$	0.992	12	0.02
Carbaryl	$y = 192.6x + 3.12$	0.931	11	0.02
Chlortoluron	$y = 122.5x + 9.69$	0.922	13	0.01
Atrazine	$y = 23.1x + 1.32$	0.977	6	0.02
Isoproturon	$y = 27.2x + 1.58$	0.961	4	0.01
Methiocarb	$y = 143.6x - 5.68$	0.938	11	0.05
Molinate	$y = 51.8x + 1.86$	0.918	6	0.01

Calibration was performed by plotting peak area versus amount injected onto the on-line precolumn system using positive- ion-mode time-scheduled SIM.

^aCV, coefficient of variation ($n = 5$) at 0.4 $\mu\text{g/l}$. Aldicarb sulfoxide, butocarboxim sulfoxide and oxamyl could not be quantified properly since their LOD were ca. 0.4 $\mu\text{g/l}$. Alachlor and metolachlor were not measured because of difficult quantitation (high variation in the tip temperature of the thermospray interface) when working at 100% of organic modifier in the LC eluent.

mode. An example reported by us recently was the determination of various acidic herbicides (2,4-D, MCPA, MCPP, and the transformation products of bentazone, among others), used in high amount in EU countries. This was achieved by preconcentrating 150 ml of estuarine waters with an on-line precolumn containing C_{18} or SDB disks followed by LC-TSP-MS (negative ions) [26]. The LODs were below 0.1 $\mu\text{g/l}$ and permitted the determination of bentazone and MCPA in real estuarine waters of the Ebro delta, at levels of 0.1–0.2 $\mu\text{g/l}$. Because of the low level of MCPA, and the lower signal under TSP-MS for bentazone, it was necessary to perform the analysis of the sample twice. In one case the $[\text{M} - \text{H}]^-$ ion of bentazone at m/z 239 was monitored whereas in the second case the $[\text{M} - \text{H}]^-$ and $[\text{M} + \text{HCOO}]^-$ ions of MCPA, at m/z values of 199 and 245, respectively, were used. In the latter case the experimental conditions were set up in order to increase the MCPA signal intensity (stem, tip, and source temperatures: 70, 160 and 200°C, respectively). Under these conditions, the signal intensity of bentazone was reduced by a factor of ten. In this way, the unequivocal determination of bentazone and MCPA at the 0.12 and 0.18 $\mu\text{g/l}$ level, respectively, was feasible in estuarine Ebro river waters samples (June 1993).

Although in the last case the technique was very useful for the confirmation of different acidic herbicides in real estuarine waters, it was still not possible to determine the acidic herbicide benazolin and the two transformation products of bentazone, 6-hydroxybentazone and 8-hydroxybentazone. For such compounds the LOD varied between 2 and 4 $\mu\text{g/l}$ when 100 ml of estuarine water samples were pre-concentrated. Although the technique – in the negative ion mode – was able to remove the humic substances present in the early eluting area of the chromatogram, problems have arisen for the ionization of various compounds at trace level.

In general, we can conclude that on-line SPE-LC-TSP-MS is a useful technique for trace analysis of pesticides in estuarine and river water samples, since removal of humic substances from the chromatographic traces is achieved. Although many pesticides present in the ongoing monitoring programmes can be analyzed, difficulties may arise for certain compounds which give difficult ionization under the techniques used, leading to worse LODs, or as a result of the high temperatures in the TSP interface that hamper the achievement of a stable signal for compounds eluting at 100% of organic modifier.

The application of this on-line methodology to the identification of photodegradation products after irradiation is new. It allows the direct analysis of the water samples after irradiation, which is a guarantee of the analytical integrity of the sample. When one applies conventional techniques, the solvent needs to be evaporated, generally to dryness, and consequently losses of compounds can take place either during evaporation or by degradation during the time of exposure. A second clear advantage when on-line SPE-LC-TSP-MS is used is that we do not need to perform any derivatization of the sample, which is also a typical procedure in photolysis experiments for the identification of the more polar transformation products. Since the technique used is very sensitive (sample enrichment by a factor of 1000 versus the conventional off-line LC technique) LODs are closer to those from GC methods, and this makes the approach particularly useful for the trace level identification of the more polar transformation products. Photolysis experiments are usually carried out at concentrations as low as 20 $\mu\text{g/l}$. Since LC-TSP-MS has sometimes given problems in the identification of unknown transformation products, the use of TSP-tandem-MS-MS enhances the structural information obtained by the LC-MS combination, providing fragmentation patterns as important as those obtained in GC-MS using the EI mode. Chiron et al. [100] confirmed that alachlor and bentazone are moderately persistent towards photolysis, and the same transformation products have been found in distilled water as in natural water. This work has expanded the list of alachlor photolysis products available to date. This is a consequence of the use of aqueous solutions and on-line SPE-LC-TSP-MS for detection purposes. Hyphenated LC-MS techniques appear to provide a powerful tool for the detection and identification of the more polar photodegradation products, and has exceeded the possibilities of GC-EI-MS in this area. The bentazone photodegradation pathway is still not well elucidated because of the likely formation of highly polar breakdown products, and their extraction from water remains a problem.

5.2.3.3. On-line SPE-LC-ESP-MS

An automated on-line SPE using OSP-2 from Merck followed by LC-ESP-MS has been applied to the determination of trace levels of the acidic herbicides and their transformation products that posed a problem with on-line SPE-LC-TSP-MS [26].

For the LC determination of the acidic herbicides benazolin, bentazone, 2,4-D, MCPA, MCPP, MCPB and 6- and 8-hydroxybentazone, the transformation products of bentazone, the acidification of the LC eluent is needed. Since in ESP-MS the ionization of the compounds takes place when ions are present in solution, as soon as the ionization is suppressed by the acidification of the eluent there is the need for the post-column addition of a neutralization buffer to avoid ion signal suppression. This method was combined with a prior automated on-line SPE step using an OSP-2 auto-sampler containing C₁₈ cartridges and was applied to the trace determination of acidic herbicides in environmental waters. The proposed method required only 50 ml of water, giving a LOD between 0.01 and 0.03 µg/l, generally employing SIM of the [M – H][–] ion (see Table 5.6). The post-column addition of neutralization buffer was required in order to form ions in solution and to facilitate a better charging of droplets. An equimolar amount of tripropylamine is therefore added, post-column, to the formic acid mobile phase. The best results were obtained with narrow bore LC columns (3 mm i.d.) using an eluent flow rate of 0.25 ml/min and the post-column addition of the buffer at a flow rate of 0.1/min. In this way the final LC carrier stream does not exceed the critical values of 0.4 ml/min which will cause a reduction in sensitivity in LC-ESP-MS [106].

To evaluate the performance of the on-line SPE-LC-ESP-MS method, river water

TABLE 5.6

CALIBRATION DATA FOR SELECTED PESTICIDES AND LOD USING TIME-SCHEDULED SIM AFTER ON-LINE SPE WITH A PRECONCENTRATION VOLUME OF 50 ml OF EBRO RIVER WATER USING LC-ESP-MS IN THE NI MODE. SPIKING LEVEL: 0.05, 0.1, 0.2, 0.8, 1 AND 5 µg/l

Analyte	Calibration equation	R ²	AV	RSD(%)	LOD (µg/l)
6-OH Bentazone	$y = 1.42 + 7.11x$	0.917	78	23	<0.01
8-OH Bentazone	$y = 1.42 + 7.11x$	0.917	76	23	<0.01
Benazolin	$y = 0.74 + 4.85x$	0.947	80	19	0.03
Bentazone	$y = 1.24 + 9.15x$	0.935	82	14	<0.01
2,4-D	$y = 0.99 + 8.04x$	0.956	74	21	0.03
MCPA	$y = 1.87 + 19.62x$	0.958	83	18	0.01
MCPP	$y = 4.71 + 31.99x$	0.942	98	22	0.01
MCPB	$y = 1.88 + 14.25x$	0.938	83	19	0.03

Calibration was performed by plotting peak area (y) versus amount injected onto the on-line precolumn system (x, µg/l) using the negative ion mode and time-scheduled SIM. Average recovery (AV) and relative standard deviation (RSD) (n = 6) at 0.2 µg/l.

samples were spiked with the mixture of seven acidic herbicides in a concentration range of 0.05–5 $\mu\text{g/l}$. The proposed on-line method required the use of 50 ml of water as the preconcentration volume, in order to achieve a LOD below 0.1 $\mu\text{g/l}$. The use of the preconcentration volume of 50 ml has an additional advantage over the 100–150 ml required in SPE-LC-TSP-MS [26] with recoveries exceeding 76% for all the compounds (see Table 5.6). When river water samples were analyzed, spiked or non-spiked with the target herbicides, the precolumns were replaced after each analysis. A typical full scan and time-scheduled SIM mode (spiking level 0.1 $\mu\text{g/l}$), is shown in Fig. 5.21 [106]. The SIM limit of detection and the calibration data for the different compounds indicated linearity from 0.05 to 5 $\mu\text{g/l}$ with LOD varying from 0.01 to 0.03 $\mu\text{g/l}$. It has been shown to be an improvement over on-line SPE-LC-TSP-MS and permitted the determination at 0.1 $\mu\text{g/l}$ levels of the acidic herbicide benazolin and of the two transformation products of bentazone that could not be determined by on-line SPE-LC-TSP-MS [26]. This review shows one of the first applications of high-flow ESP to the determination of pesticides in water, with an increase in the LOD compared to TSP of ca. 100 times. So far, ESP has been mainly used for the characterization of high molecular weight compounds but it has scarcely been used for low molecular weight compounds.

The proposed on-line SPE-LC-ESP-MS permitted the determination of benazolin and the two transformation products of bentazone, 6- and 8-hydroxybentazone at environmental water concentrations below 0.1 $\mu\text{g/l}$. This is an improvement since previously no analytical method was available for their determination at such concentration levels. The whole method only requires 50 ml of water and, for the first time, an automated preconcentration system, OSP-2, was coupled on-line with high flow LC-ESP-MS which has been demonstrated to be a powerful technique in environmental water analysis. Another advantage of the present system is that the use of ESP-MS provides much better structural information than does TSP-MS, being a useful technique for identification of unknown acidic herbicides in estuarine water samples. In this way it has been shown that the present system can be used as an “alarm” for the identification of unknown acidic herbicides in estuarine water samples at levels as low as 3 $\mu\text{g/l}$ (see Fig. 5.21) using full scan conditions. The possibility of inducing fragmentation at higher extraction voltages has made it possible to distinguish the two isomeric transformation products of bentazone usually found in river water samples and to make unequivocal confirmation of the presence of 8-hydroxybentazone in estuarine waters.

5.2.3.4. On-line SPE-LC-APCI-MS

Liquid chromatography coupled to atmospheric pressure, chemical ionization mass spectrometry, with positive and negative modes of operation, was used for the determination of several organophosphorus pesticides: mevinphos-*cis* and -*trans*, dichlorvos, azinphos-methyl, azinphos-ethyl, parathion-methyl, parathion-ethyl, malathion, fenitrothion, fenthion, chlorfenvinphos and diazinon. This method was

combined with a prior automated on-line liquid-solid extraction step using an OSP-2 autosampler containing C_{18} cartridges and was applied to the trace determination of organophosphorus pesticides in ground water. The proposed method required only 100 ml of water to give a limit of detection between 1 and 25 ng/l in both modes of ionization, generally employing selected-ion monitoring of the group-specific fragment of the organophosphorus pesticides [107].

Table 5.7 shows the calibration equations and LOD achieved by preconcentrating 100 ml samples containing organophosphorus pesticides. The sample cone needs to be cleaned frequently. This operation is matrix dependent; with ground water samples, the cleaning operation was done routinely every three to four days whereas with estuarine water 2 days is generally the maximum operational period to achieve the better performance.

Typical chromatograms obtained under PI- and NI-mode using full-scan and SIM conditions, are shown in Fig. 5.22 for the analysis of 100 ml water samples spiked at 1 $\mu\text{g/l}$ using full scan conditions (A) and time-scheduled SIM under PI mode (B) and time-scheduled SIM under NI mode (C). The sensitivities with the two modes of operation were not so different, and this is one of the best achievements of this technique as compared to ESP. In general, the parathion group was determined better under the NI mode of operation whereas dichlorvos and diazinon were detected better under the PI mode. An interesting feature of the present system is that it can be operated under full-scan conditions for an "alarm" when pesticides are present in ground water samples. These levels are generally set at 2 $\mu\text{g/l}$, and from the LC-APCI-MS traces in Fig. 5.22A, it is clear that this technique can be applied for the identification of unknowns in water samples.

By the use of automated on-line LSE followed by LC-APCI-MS, the determination of a variety of organophosphorus pesticides in 100 ml of ground water samples was feasible with LODs varying from 1 to 25 ng/l in both modes of operation. The method leads to an unequivocal confirmation of the organophosphorus pesticides and it is more powerful, for many of the compounds studied, than is ESP-MS which only showed good sensitivity in the PI mode of operation. The structural information obtained using APCI-MS under NCI has many similarities with conventional NCI-MS and it makes the present approach useful for trace determination and the unequivocal confirmation of organophosphorus pesticides in environmental waters. Considerable fragmentation of the analytes, together with molecular weight information was achieved.

The present system can be used to provide alarm information on the presence of organophosphorus pesticides in water samples. With preconcentration of only 100 ml of water, the system is able to identify all the analytes at the 2 $\mu\text{g/l}$ level, using both modes of operation and full-scan conditions. In this way, either on-line SPE-LC-APCI-MS or SPE-LC-ESP-MS are good alternatives to the screening of unknown analytes in water samples by preconcentrating only 50–100 ml of water. These new techniques represent clear improvements over previous LC-MS methods

TABLE 5.7

CALIBRATION DATA OBTAINED WITH LC-APCI-MS IN TIME-SCHEDULED SIM-PI MODE FOR THE STUDIED PESTICIDES (SPIKED FROM 0.0125 TO 1 ng/ml) AFTER ON-LINE PRECONCENTRATION OF 100 ml OF WATER

Compound	Calibration equation	R^2	Linear range (ng/ml)	LOD (ng/l)	LOQ (ng/l)
Mevinphos- <i>cis</i>	$y = 4838 + 1024874x$	0.995	0.0125–1	2	10
Mevinphos- <i>trans</i>	$y = 6254 + 1014044x$	0.996	0.0125–1	2	10
Dichlorvos	$y = 944 + 562748x$	0.993	0.0125–1	6	12
Azinphos-methyl	$y = 4304 + 22397x$	0.999	0.0250–1	12	20
Parathion-methyl	$y = 965 + 412492x$	0.999	0.0125–1	9	12
Malathion	$y = 63091 + 640908x$	0.992	0.0125–1	3	10
Fenitrothion	$y = 4318 + 385572x$	0.998	0.0125–1	9	12
Azinphos-ethyl	$y = -1880 + 293380x$	0.994	0.0250–1	14	25
Parathion-ethyl	$y = 4565 + 239806x$	0.999	0.0250–1	15	25
Fenthion	$y = -3390 + 472773x$	0.998	0.0250–1	10	20
Chlorfenvinphos	$y = -6816 + 1214890x$	0.998	0.0125–1	1	5
Diazinon	$y = 5567 + 1354616x$	0.998	0.0125–1	2	10

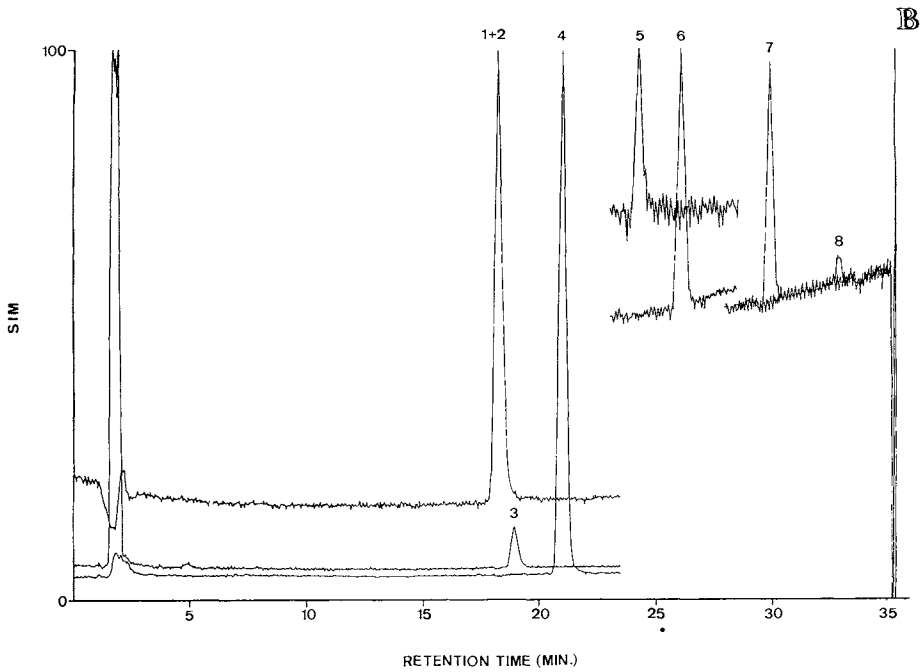
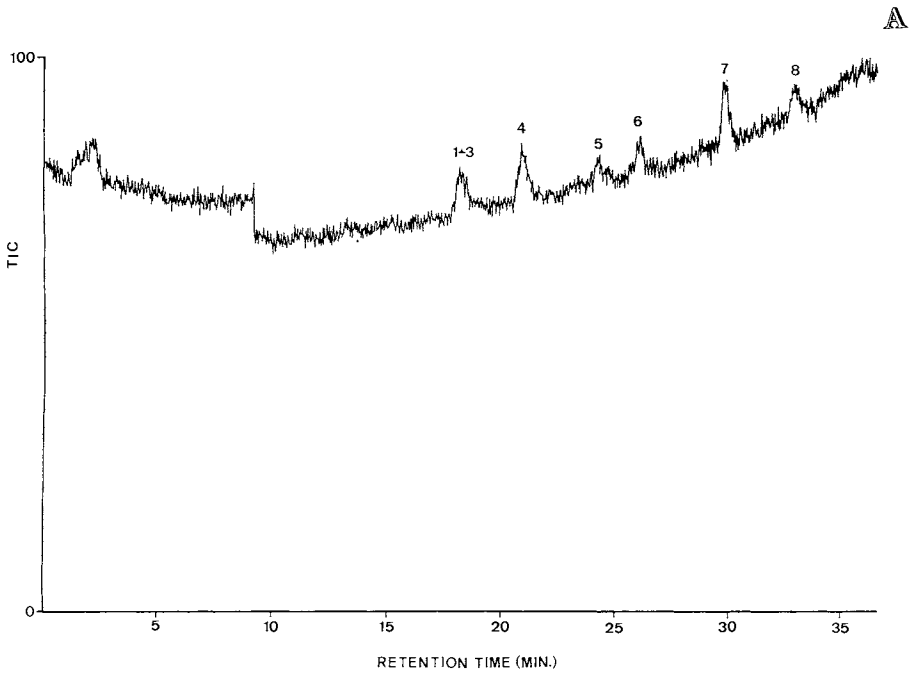
LOD were calculated by using a signal-to-noise ratio of 3–4 the ratio between the peak intensity and the noise). LOD repeatability ($n = 5$) varied between 28 and 40%. LOQ was obtained from LOD (1.3–5 times). LOQ repeatability ($n = 5$) values varied from 17 to 25%.

since, in addition to the sensitivity enhancement, there is also the possibility of achieving structural information.

5.2.4. On-line coupled reversed-phase liquid chromatography and liquid chromatography

The main features of on-line systems with precolumns have been highlighted in the above section. It was shown that the selectivity can be enhanced by using selective trapping materials or by using selective detection modes. Another way towards more selectivity is to replace the precolumns by a full-size analytical column. This approach was developed for the determination of very polar pesticides, since the

Fig. 5.21. On-line SPE, using OSP-2 autosampler followed by LC-high-flow ESP-MS using the NI mode, of a 50 ml river water sample spiked with (A) 3 $\mu\text{g/l}$ under full scan, and (B) 0.1 $\mu\text{g/l}$ under time scheduled SIM, of (1) 8-hydroxybentazone, (2) 6-hydroxybentazone, (3) benazolin, (4) bentazone, (5) 2,4-D, (6) MCPA, (7) MCPP, (8) MCPB. Ions monitored correspond to $[M - H]^-$. Gradient elution was accomplished by using an eluent containing 20% of solvent A (methanol) and 80% of solvent B (water pH 2.9 with formic acid) to 80% A–20% B in 30 min at a flow rate of 0.25 ml/min, then back to initial conditions in 5 min. The analysis involved a Lichrocart cartridge column (125 \times 3 mm i.d.) packed with Lichrospher 60RP select B material of 5 μm particle size from Merck (Darmstadt). A post-column addition of 0.1 ml/min of tripropylamine (4g/l methanol) was carried out by using a 64-high pressure pump from Knauer (Bad-Homburg) and a Valco tee. From Ref. [106] with permission.



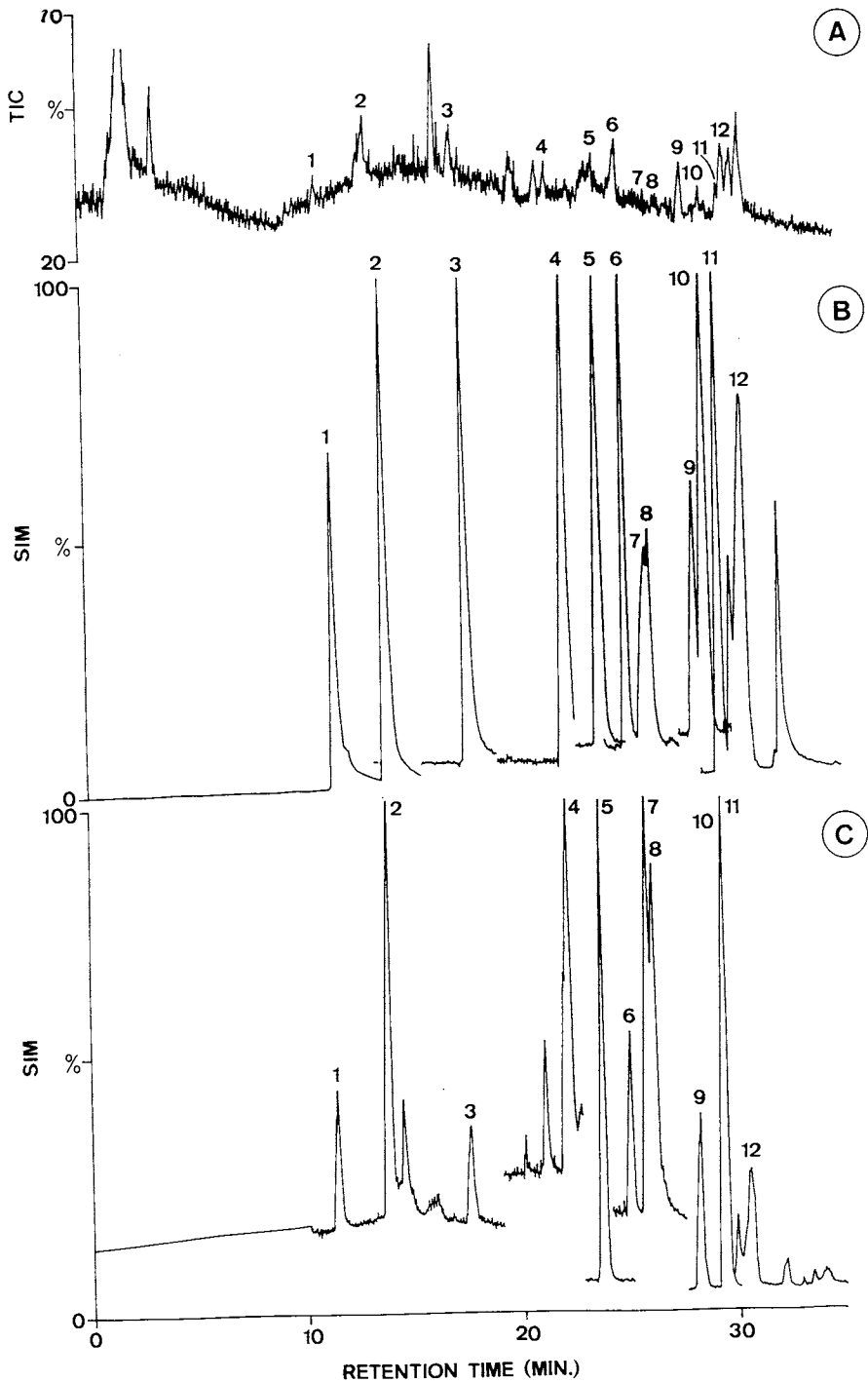
analyst of surface and ground water is confronted with an excess of polar interferences such as humic and fulvic acids, making the determination of very polar pesticides difficult or impossible [108]. The principle is easy; the sample is percolated through the first column and, in comparison to automated on-line sample enrichment on precolumns, one of the most favourable aspects of automated sample processing using LC-LC is the utilization of the separation power of the first column. As shown in Fig. 5.23a, it offers the possibility of removing a large excess of early-eluting polar interferences and then transfers a pre-separated band (A) containing the analytes of interest to the second LC column. One advantage can be seen in Fig. 5.23b for the rapid analysis of methabenzthiazuron at a level of 0.1 $\mu\text{g/l}$ from only 4 ml of ground water sample [109]. The method was applied to the determination of very polar analytes such as chloroallyl alcohol, methyl isocyanate and the main carbamate metabolite, ethylenethiourea [110–112]. Another relevant application deals with the determination of acidic pesticides such as bentazone and glufosinate [113]. Multi-residue approaches were also developed but required larger transfer volumes, thus providing lower attainable selectivity. The method has also been used for the determination of various herbicides in well-waters and for the simultaneous analysis of eight chlorophenoxy acids, dicamba and bentazone in ground water and surface water, but an additional clean-up over silica SPE was required [114,115].

5.3. ON-LINE TECHNIQUES WITH SEPARATION BY GAS CHROMATOGRAPHY

The GC technique offers the unique advantage of a high separation power and a range of very sensitive and selective detection modes, including easy interfacing with MS. In environmental analysis, the sample pretreatment for GC is almost an off-line procedure, with the inherent limitation of a final extract between 50 and 500 μl , whereas the injection volume is often 1–5 μl . Although in recent years the increasing use of on-column injection in combination with retention gaps and partial concurrent solvent evaporation has allowed a useful increase of the injection volume [116–119], the direct introduction of a polar solvent containing traces of residual water still presents severe problems.

The coupling of liquid and gas chromatography on-line has become more important in analytical chemistry, but almost all the applications consist of a normal-phase pre-separation via a LC column and the subsequent separation of a heart-cut by GC, as can be seen in some recent reviews [120–127]. Some applications utilized this

Fig. 5.22. On-line LSE using OSP-2 autosampler, followed by LC-APCI-MS of 100 ml of ground water samples spiked with 1000 ng/l of organophosphorus pesticides under PI mode and full scan conditions. (A) Time-scheduled- SIM; (B) SIM using NI mode of operation, of (1) mevinphos *cis*; (2) mevinphos *trans*; (3) dichlorvos; (4) azinphos-methyl; (5) parathion-methyl; (6) malathion; (7) fenitrothion; (8) azinphos-ethyl; (9) parathion-ethyl; (10) fenthion; (11) chlorfenvinphos; and (12) diazinon.



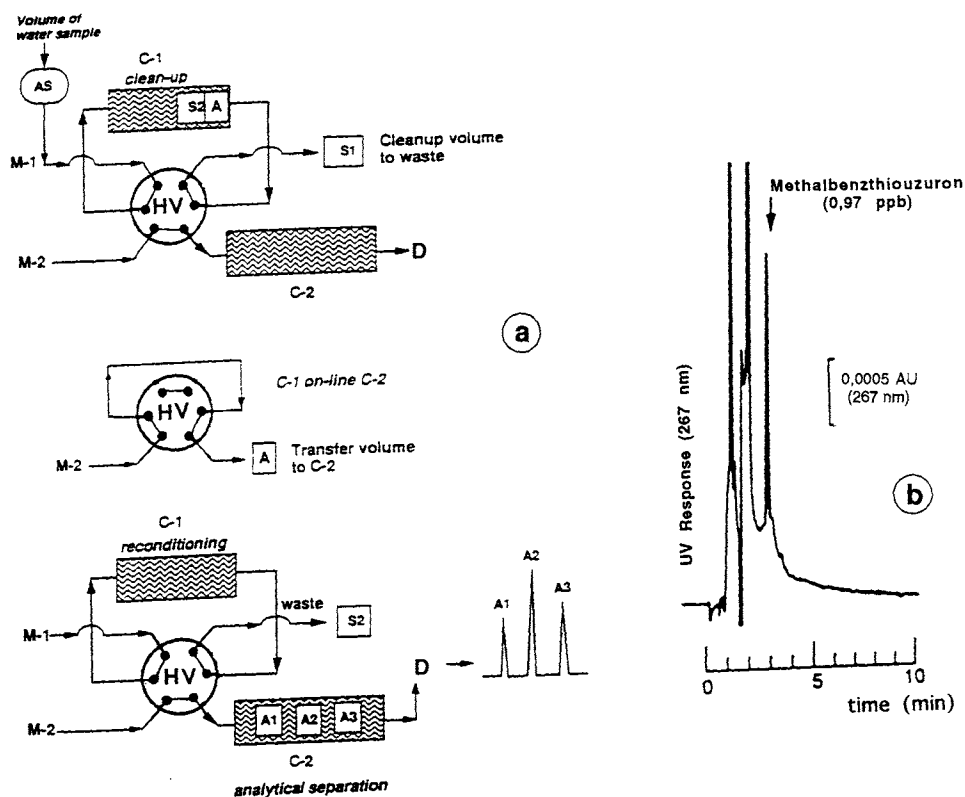


Fig. 5.23. (a) Schematic representation of separation procedure involved in coupled-column RPLC. As, autosampler; C-1, C-2, first and second separation columns, respectively; M-1, M-2, mobile phases; S1, S2, interferences; A, target analytes; HV, high-pressure valve; D, detector. From Ref. [108]. (b) Direct coupled-column RPLC-UV (267 nm) analysis (4.0 ml injection) of a spiked ground water sample (methalbenzthiazuron, 0.97 $\mu\text{g/l}$). LC conditions: C-1 and C-2, 3- μm Microspher C_{18} (100 \times 4.6 mm i.d.); M-1 and M-2, acetonitrile–water (45:55, v/v) with a flow rate of 1 ml/min; clean-up volume, 5.9 ml; transfer volume, 0.45 ml. From Ref. [109].

coupling for the analysis of pesticides in sediments [128], or in fat-matrices [129], or for an automated clean-up step after a micro liquid-liquid extraction step [130,131].

5.3.1. On-line solid-phase extraction and gas chromatography (SPE-GC systems)

The trace-enrichment of pesticides from aqueous samples involves the coupling of a reversed-phase type of liquid-chromatography with GC, and is not straightforward since water should not enter the GC part of the system. An intermediate step involv-

ing an on-line liquid-liquid extraction with the addition of organic solvent and a phase separator has been proposed, but the apparatus is rather complicated [132, 133].

For the numerous pesticides which are directly amenable to GC without prior derivatization or conversion, on-line preconcentration and GC should be the method of choice, and some work has been directed towards this. Its feasibility was first demonstrated in 1987 for the trace-level determination of chlorinated pesticides and PCBs in water samples, as shown in Fig. 5.24 [134]. The analytes were extracted on a 4×1 mm i.d. micro-precolum and the precolumn was dried with a stream of ni-

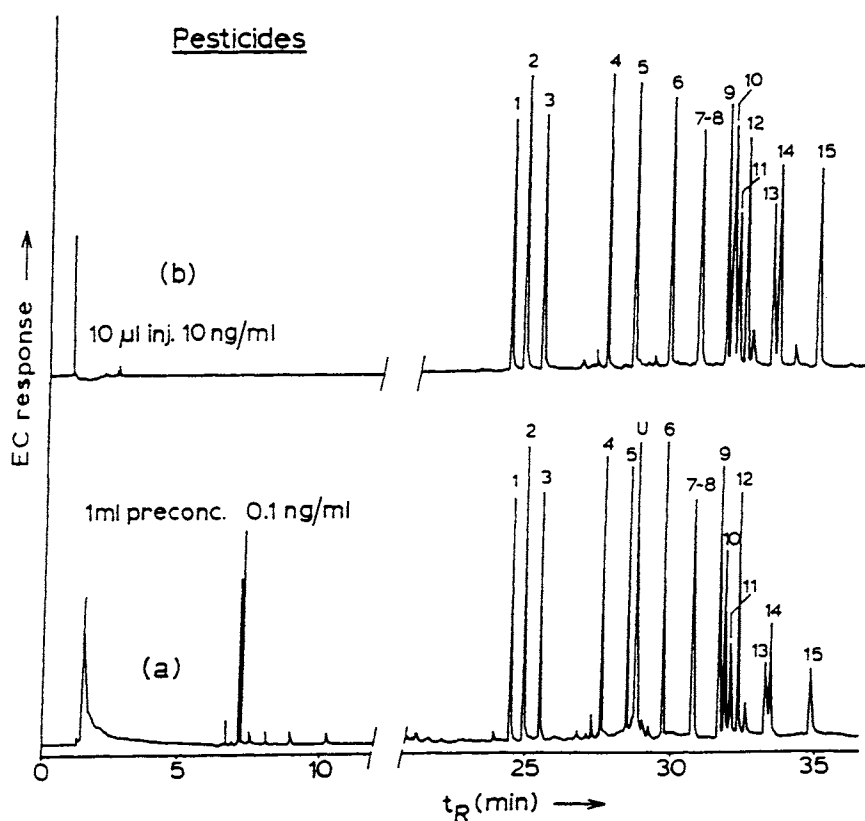


Fig. 5.24. (a) GC chromatogram of fifteen pesticides in water after trace enrichment. Sample: 1 ml trace enrichment of 0.1 ng/ml (100 ppt) of each pesticide in 10% methanol and elution by $85 \mu\text{l}$ hexane. (b) A reference chromatogram: $10 \mu\text{l}$ injection of 10 ng/ml of the pesticides in hexane. Compounds: (1) α -HCH; (2) HCB; (3) γ -HCH; (4) heptachlor epoxide; (5) aldrin; (6) heptachlor; (7) *o,p'*-DDE; (8) endosulfan; (9) *p,p'*-DDE; (10) dieldrin; (11) *o,p'*-DDE; (12) endrin; (13) *p,p'*-DDE; (14) *o,p'*-DDT; (15) *p,p'*-DDD. U, unknown compound. GC conditions: column 25 m CP Sil 5 CB and 5 m retention gap; ECD detection mode. From Ref. [134] with permission.

trogen before the analytes were desorbed by hexane. The use of the sensitive ECD detector allowed the preconcentration of only 1 ml for detection limits as low as a few ng/l. Although this example has clearly shown the potential of the method with the handling of sample volumes of the order of 1 ml, one must admit that the on-line coupling of SPE with GC has not yet become as routine an application as on-line SPE-LC. The simple reason is that in SPE-LC systems, the presence of water is not a problem whereas in SPE-GC one needs an interface which enables the direct coupling of an aqueous reversed-type LC part with the strictly non-aqueous GC part.

Extensive work has been carried out during the last 5 years, and the increasing number of published works has demonstrated the versatility of on-line SPE-GC. Recent reviews have reported on the coupling of the SPE enrichment of aqueous samples with GC [3,135–137].

5.3.1.1. *On-line SPE-GC arrangement*

The apparatus which had given the results presented in Fig. 5.24 was modified in order that polar solvents could be used for the desorption step, because hexane should not elute polar pesticides. A first modification of the interface was made for the introduction of the desorption solvent into the GC system by adding a splitter located at the entry of the GC. This should allow the removal of the evaporated solvent and cold-trapping of the analytes [138].

Much effort has been devoted by Vreuls to the on-line coupling of the SPE treatments with aqueous samples and GC [139]. A totally automated on-line set-up, with a Prospekt unit for the solid-phase extraction of water samples is shown in Fig. 5.25 [140]. Water samples are preconcentrated on a 10×1.5 mm i.d. precolumn packed with C_{18} silica or apolar copolymer PLRP-S, i.e., the same precolumns as those used for the on-line coupling with LC. The sequence is the same: (i) activation with methanol, and (ii) loading the aqueous sample and flushing with water. Of course, since the sample volumes are typically 1–10 ml, the problem of analyte breakthrough does not exist here. Then, the precolumn is dried by a stream of nitrogen. A syringe pump is used in position 4 for delivering the desorption solvent. The analytes are transferred to the GC by a volume of 50–100 μ l of polar solvent at a low flow rate, typically 50–200 μ l/min. Different polar solvents were studied and ethyl acetate was selected for two reasons. First it can push out to waste any water remaining in the capillaries and in the precolumn, before desorption and transfer. Secondly, ethyl acetate as solvent permits the removal of traces of water by forming an azeotrope with it which will be completely eliminated during solvent evaporation in the retention gap [141,142]. However, further experiments have shown that it is necessary to remove traces of water in the ethyl acetate before transfer in order to avoid destruction of the deactivation of the retention gap which can generate active sites responsible for peak-tailing of high-boiling analytes [120]. A first solution to this problem consisted in purging the SPE precolumn with a stream of nitrogen at 40 ml/min and

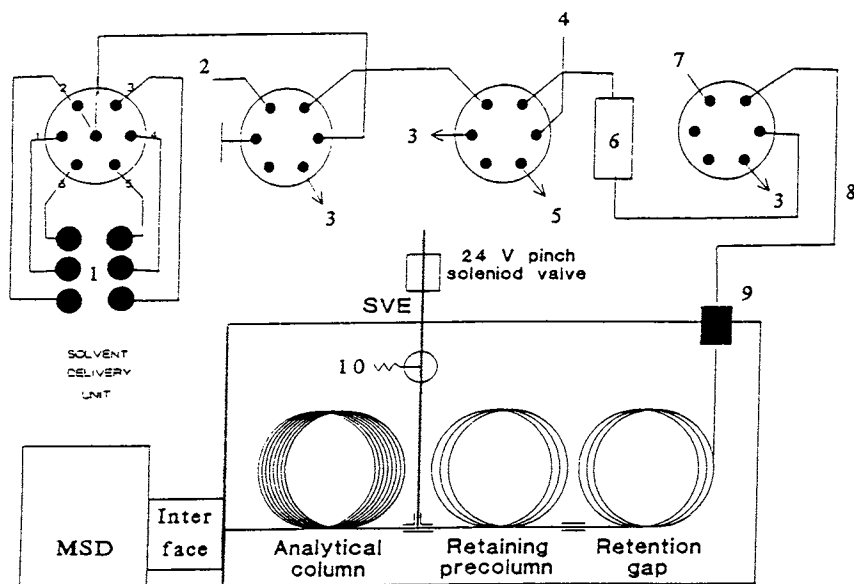


Fig. 5.25. Schematic diagram of an on-line SPE-GC-MS system containing three six-port switching valves and a solvent delivery unit (SDU) system for the automated SPE sequence, drying with nitrogen, desorption with ethyl acetate and MS coupling. The numbers of the different parts are: (2) nitrogen entry; (3) waste; (4) syringe pump; (6) precolumn; (7) loop injection; (9) on-column injector; (10) solvent vapour exit. From Ref. [140] with permission.

at ambient temperature during 25–30 min which allowed good removal of traces of water without loss of compounds [143].

When large volumes are introduced into the capillary column, the broad peaks of the analytes have to be reconcentrated in order to be separated with good peak shapes. The preconcentrating effect can be obtained simply by the solvent effect for volatile analytes or by the so-called phase-soaking and phase-ratio effect for less volatile analytes, as described by Grob and Schilling [144,145]. Even when using injection systems which allow concurrent solvent evaporation, Dolecka et al. [146] noticed that non-volatile analytes could still be separated with good peak shapes if they are introduced at a flow rate below the evaporation rate of solvent. The system described in Fig. 5.25 allows a high evaporation rate in the retention gap via a split outlet between the retention gap and the capillary column. It is also important that the ethyl acetate is introduced at a constant flow rate and without pulsation, which is easily obtained via a syringe pump.

The residual water is certainly one main problem to be solved because the drying step with a nitrogen stream is time-consuming. It was noticed that the polymeric sorbent PLRP-S was dried more rapidly than C₁₈ silica: this latter packing shows more affinity for water because the matrix contains residual silanol groups. Another

option for removing traces of water is to introduce a small cartridge packed with a drying agent, i.e., anhydrous sulfate or silica. The cartridge is placed between the precolumn and the GC system and water is removed from the ethyl acetate before its introduction into the GC system. It was shown that either a 30 min nitrogen purge or the use of a drying agent allowed the analysis of a series of *S*-triazine herbicides and several rather volatile test compounds with good repeatability and with negligible analyte losses even at the sub- $\mu\text{g/l}$ level [143,147–149]. After transfer, regeneration of the drying cartridge by electric heating and drying allowed its re-use for about 20 runs [143]. The use of extraction disks in special precolumns (those designed for on-line SPE-LC systems) allowed a reduction in the time required for drying the precolumn with a stream of nitrogen to 10–15 min [148].

5.3.1.2. Applications and detection limits with various detection modes

The method described above was successfully applied to the determination of organophosphorus pesticides using an on-line cartridge packed with three 0.5 mm thick, 4.2-mm diameter membrane- extraction disks, a drying step using a stream of nitrogen during 10–15 min at ambient temperature, desorption by ethyl acetate introduced directly into the retention gap and final thermoionic detection [147]. Figure 5.26 shows the LC-GC-NPD chromatogram of 2.5 ml of river Garonne water, non-spiked and spiked with a series of organophosphorus pesticides at concentrations between 115 and 180 ng/l. It is clear that detection limits of 10–30 ng/l are easily achieved in this surface water sample, owing to the sensitivity and selectivity of the thermoionic detector. Samples of Rhine water were also analyzed [147] and, although more compounds were co-extracted, the detection limits were still as low as 50–100 ng/l. The method was reproducible with average RSD between 2 and 4%. Linearity was obtained for the whole procedure over the range 0.06–3 $\mu\text{g/l}$.

A comparison between the three detectors, flame ionization (FID), nitrogen-phosphorus (NPD) and flame photometry (FPD), has been presented by Picó et al. [148,149] with the determination of several triazines, organophosphorus pesticides, and sulfur-containing compounds in tap-water using polymer-packed precolumns, drying via a silica cartridge, and desorption with ethyl acetate. The same retention gap was used for the analysis of ca. 100 samples without significant deterioration of the chromatographic peaks. With 10 ml samples of tap-water, the analyte recoveries were at least 72%. Detection limits below 0.1 $\mu\text{g/l}$ are obtained for all the analytes in tap water with any of the three detectors, although NPD and FPD were more selective. The difference in selectivity and sensitivity between the FID and NPD modes is illustrated in Fig. 5.27. The spiking level with the FID mode is 0.3 $\mu\text{g/l}$ whereas with the NPD mode it is 0.1 $\mu\text{g/l}$ for triazines and 0.03 $\mu\text{g/l}$ for OPPs.

Atomic emission detection (AED) was also investigated, with direct injections of 100 μl of sample solutions in ethyl acetate, via a loop-type interface [150]. A series of organophosphorus compounds was selected as test analytes and monitored using the carbon, sulfur, nitrogen, chlorine, bromine and phosphorus channels. In this ex-

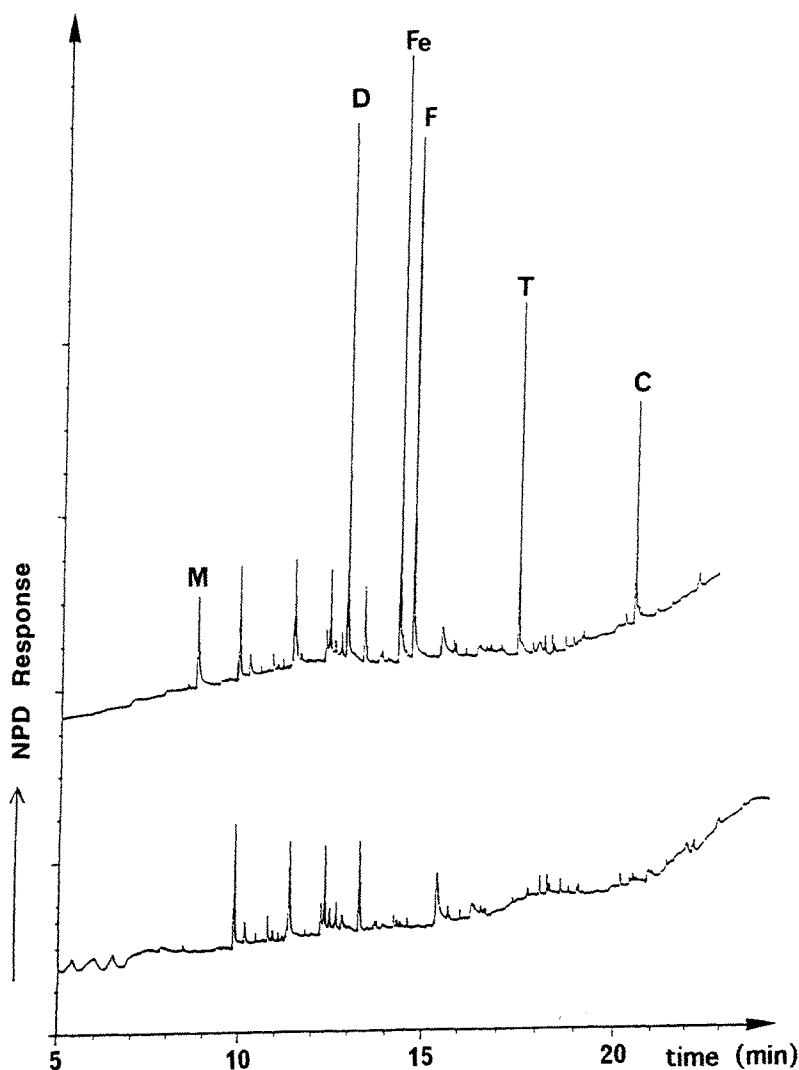


Fig. 5.26. SPE-GC-NPD chromatogram of 2.5 ml of (A) river Garonne water and (B) river Garonne water spiked with six OPPs. Concentration in ppt: mevinfos (M) 185; diazinon (D) 135; fenitrothion (Fe); 180; fenthion (F) 170; triazofos (T) 135; coumaphos (C) 115. From Ref. [147] with permission.

periment, GC-AED was combined off-line with solid-phase extraction. The results show that the analytical potential of the system should be compatible with an on-line system.

The potential of the LC-GC-MS was also investigated, using a polymeric precolumn and nitrogen drying [151–153]. Because of the wide variety of contaminants present in surface waters, there is a need for analyzing both target and non target analytes, and the two options were presented.

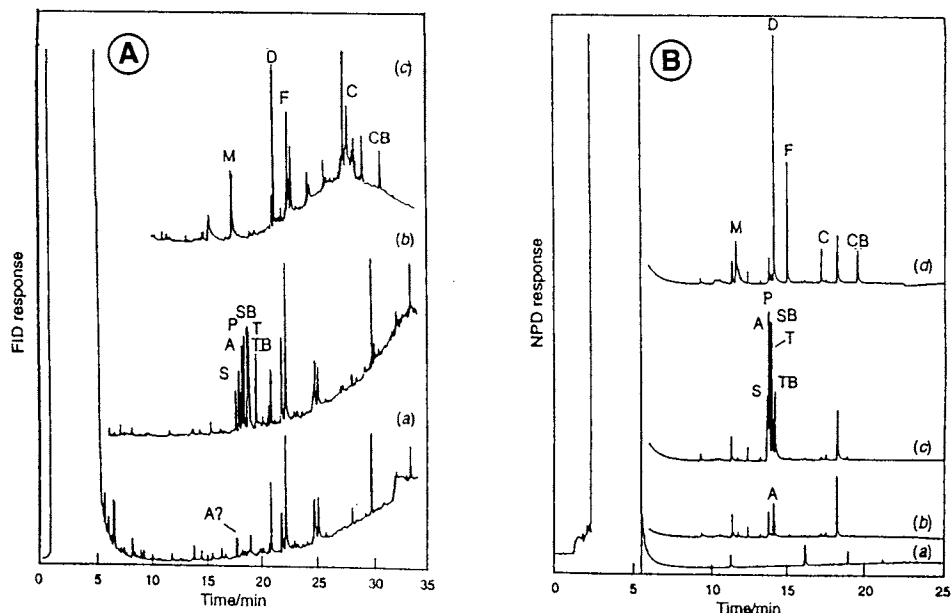


Fig. 5.27. SPE-GC-FID: (A) Chromatograms after preconcentration of 10 ml of (a) Amsterdam drinking water, and drinking water spiked with (b) triazines (S, simazine; P, propazine; SB, secbumeton; T, trietazine; TB, terbutylazine) and (c) OPPs (M, mevinphos; D, diazinon; F, fenitrothion; C, coumaphos; CB, carbophenthion). Spiking level. $0.3 \mu\text{g/l}$. (B) SPE-GC-NPD chromatogram after preconcentration of (a) HPLA-grade water, (b) Amsterdam drinking water, (c) drinking water spiked with triazines (spiking level $0.1 \mu\text{g/l}$) and (d) OPPs (spiking level $0.3 \mu\text{g/l}$). From Ref. [149] with permission.

The on-line LC-GC-MS system was used for the identification of unknown pollutants at trace-level in a Rhine water sample previously spiked with a standard mix of 162 environmental contaminants at $1 \mu\text{g/l}$ [153]. From 1 ml samples, non-target compounds could be analyzed and identified in the full-scan acquisition mode, all compounds eluting from the column being recorded in the total-ion-current (TIC) chromatogram. The detection limits were between 10 and 100 pg. Target analysis at the ng/l level is preferably done by recording mass traces in the MID mode. Figure 5.28 shows the MID chromatogram corresponding to 1 ml of a sample of river Meuse water in which atrazine and simazine were detected. When working in the MID mode and using a sample volume of 1 ml for trace enrichment, the limit of detection was about 5 pg for triazines or 5 ng/l in surface waters.

When both non-target and target analytes are the goals of an analysis, it is preferable to work in the TIC mode, and the sensitivity of the system in terms of detectability was improved by increasing the sample volume to 10 ml. Figure 5.29 presents the TIC chromatogram obtained after trace enrichment of 10 ml of river Meuse water. The extracted ion chromatograms of simazine and atrazine indicated a higher detection limit (30 pg for both tested triazines) than that observed in Fig. 5.28, but

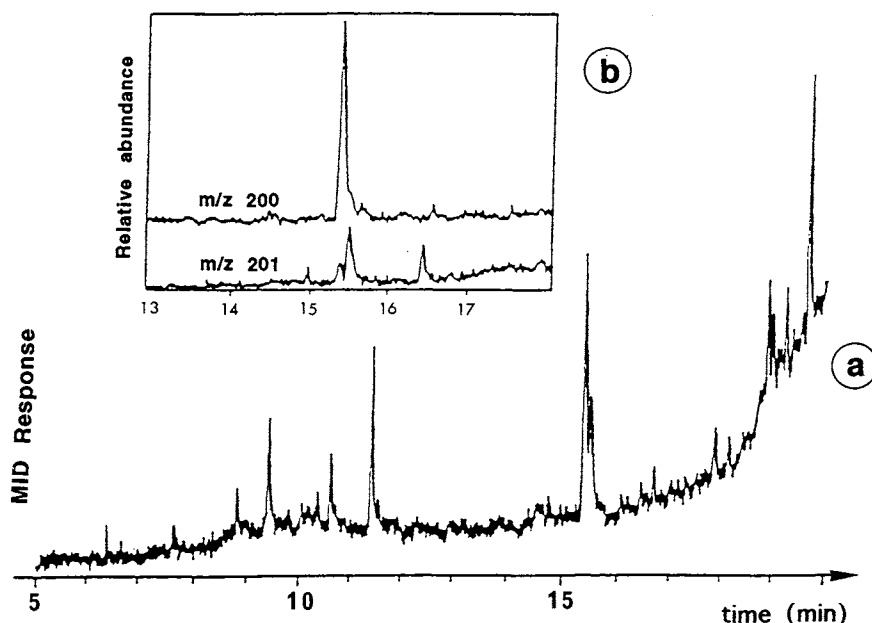


Fig. 5.28. (a) Multiple-ion detection in on-line LC-GC-MS of 1 ml of river Meuse water m/z values selected: m/z 200, 202, 215 and 217 for atrazine and m/z 186, 18, 201 and 203 for simazine. (b) Insert shows reconstructed ion traces of the 13–18 min part of the chromatogram: m/z 200 for atrazine (upper chromatogram) and m/z 201 for simazine (lower chromatogram). From Ref. [153] with permission.

correspond to a lower concentration of 3 ng/l in the real sample, since the sample volume was 10 times higher.

5.3.2. On-line supercritical fluid extraction and gas chromatography

Supercritical fluid extraction (SFE) allows a part of a sample to be transferred to the next separation step, depending on the density of the fluid, the temperature and the addition of a modifier. The sample transfer from the supercritical state is more easily adaptable to coupled systems than from a sample in the liquid state, owing to the high volatility of the fluid at atmospheric pressure. Two modes are available in commercial instruments for the coupling of SFE to GC [154]. One is the robotic mode, where the extract is transferred from the collector through robotic interactions, and the other one is the on-line mode. A simple interface has been described for the on-line transfer [155]. After SFE, the extracted analytes are brought to the restrictor, precipitated at the lowered pressure and temperature and collected in the cold trap close to the head of the GC column. Then connection to the GC column occurs and the analytes are transferred by heating the trap. Care has to be taken in this transfer in order to inject a sharp plug and avoid band broadening. The optimization of the different parameters has been described [156,157] and is not detailed here.

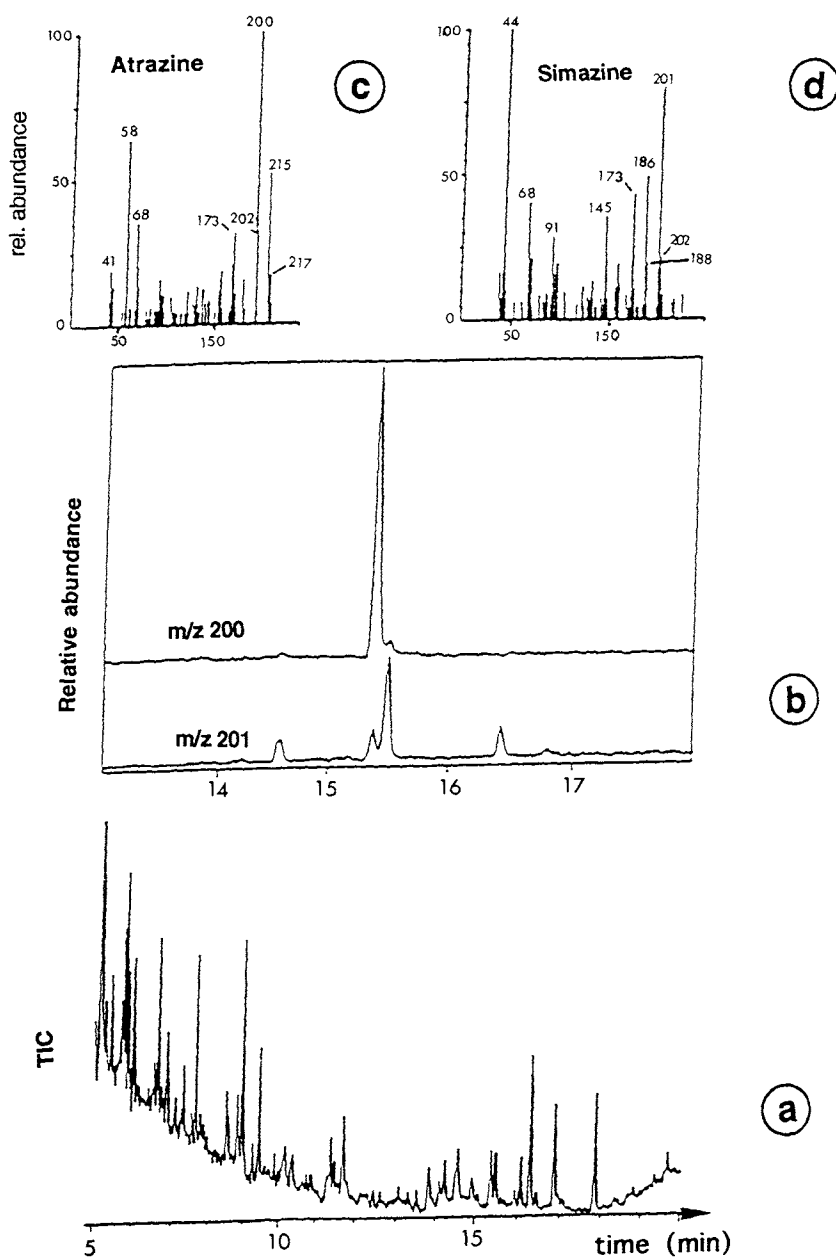
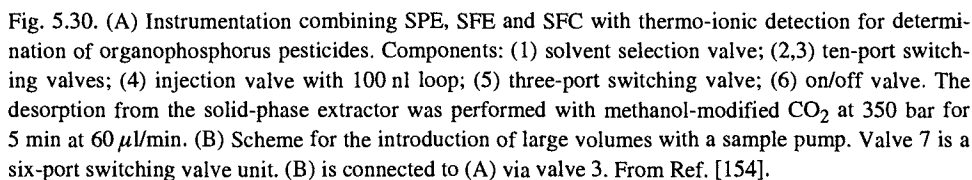
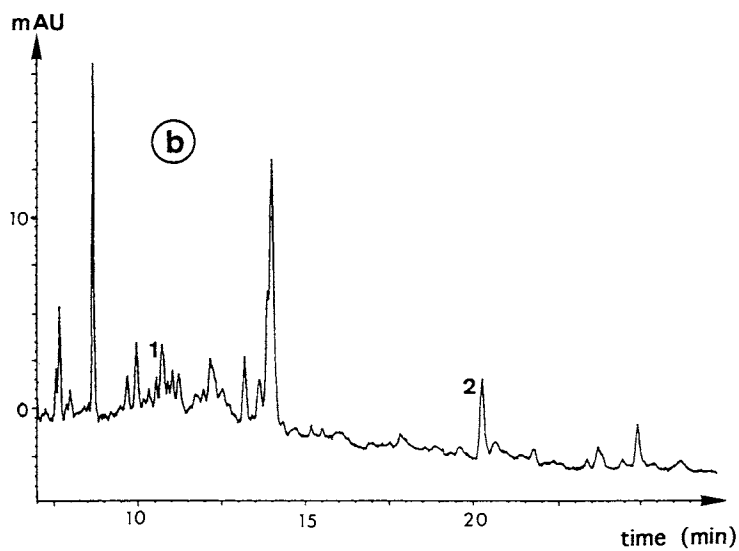
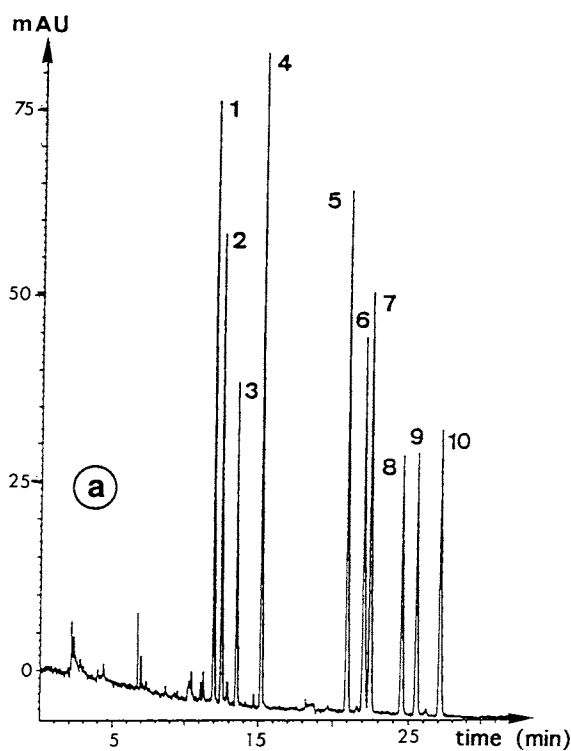


Fig.5.29. (a) TIC trace obtained after on-line LC-GC-MS of 10 ml of river Meuse water with full-scan acquisition over the mass range m/z 35–385. (b) Reconstructed ion trace for atrazine (m/z 200) and for simazine (m/z 201). Mass spectra recorded for (c) atrazine peak and (d) simazine peak. From Ref. [153] with permission.



Most applications using on-line SFE-GC deal with the handling of solid samples for the determination of hydrocarbons. Few applications with the handling of aqueous samples have been described. The most relevant example is the determination of organochlorine pesticides which were concentrated on a C₁₈ cartridge, then further desorbed by SFE, and analyzed on-line by GC-ECD [158].



5.4. ON-LINE SOLID-PHASE EXTRACTION, SUPERCRITICAL FLUID EXTRACTION, AND SUPERCRITICAL CHROMATOGRAPHY

The on-line coupling of SPE-SFE-SFC has been described for the trace determination of pesticides in aqueous samples [159]. The system shown in Fig. 5.30 allows the extraction of pesticides from large volumes on a C_{18} precolumn via the sample pump (Fig. 5.30B). The on-line SFE-SFC system is described in Fig. 5.30A and required a SFC pump as well as a LC pump for reproducible addition of the modifier (pure methanol or methanol–water in the example). Then, after drying with nitrogen, the desorption from the SPE cartridge was performed with methanol-modified CO_2 at 150 bar. A thermoionic detector allowed detection limits between 0.1 ng/l and 1 μ g/l depending on the size of the precolumn.

Another relevant example shows the selectivity given by the SFE-SFC for the determination of more polar pesticides, such as phenylureas using DAD detection [160]. In comparison to classical SPE-LC-DAD, the advantages are speed of analysis and desorption selectivity. A mixture containing ten phenylureas was separated by packed-column SFC operated at 40°C in order to avoid thermodegradation of the phenylureas [161,162]. The SFC separation required the addition of methanol to the CO_2 . The arrangement is roughly similar to that described in Fig. 5.30. The SPE step was optimized using C_{18} and PLRP-S cartridges. In the sampling configuration used, the cartridge was conditioned with methanol and water, then 10 ml of sample was added followed by 2 ml of double distilled water, before being dried with a stream of nitrogen. Desorption was performed using 1% methanol in carbon dioxide at 100 bar and at a flow rate of 2 ml/min for 1 min. After the desorption, the valves were repositioned to load a new samples since sample handling occurred during the SFC analysis time. The critical factors in the procedure were the drying time, the desorption time, and the selection of C_{18} silica versus SDB material. A 15-min drying period was required to obtain good shapes for the peaks, with the handling of 10 ml of LC-grade water spiked with a mixture of ten phenylureas. The desorption time required was at least 1 min to obtain good recoveries. The authors show that, using a 20 \times 2.1 mm i.d. precolumn, the C_{18} cartridges gave low recoveries for polar analytes such as de-isopropylatrazine even with 10 ml samples, and that for such compounds SDB sorbents gave excellent recoveries. Figure 5.31a shows the chromatogram ob-

Fig. 5.31. Chromatograms obtained from on-line SPE-SFC-DAD system. (a) Obtained with the handling of 10 ml water sample spiked with 10 μ g/l of ten phenylureas and (b) determination of pesticides in water from the river Leie with identification of atrazine and isoproturon. From Ref. [160]. SPE through a 20 \times 2.1 mm i.d. precolumn packed with C_{18} silica, with a drying time of 15 min and a desorption time of 1 min at a flow rate of 2 ml/min. Separation using a packed-column (25 \times 4.6 mm i.d. LiChrospher silica 60, 5 μ m) operated at 40°C; mobile phase CO_2 with methanol at a flow rate of 2 ml/min from 100 to 250 bar at 5 bar/min. Methanol concentration from 1 to 15% at 0.5%/min. Solutes: (1) monolinuron; (2) metobromuron; (3) linuron; (4) methabenzthiazuron; (5) isoproturon; (6) fenuron; (7) chlorotoluron; (8) diuron; (9) chloroxuron; (10) metoxuron.

tained under optimized conditions. The whole procedure was applied to the determination of seventeen pesticides in river water. In Fig.5.31b, atrazine and isoproturon were detected at concentrations of 0.54 and 2.41 $\mu\text{g/l}$, respectively, from only 10 ml samples. One interesting point is that no humic interferences are detected with the DAD. The sensitivity can be increased greatly by using the new HSA/SDB polymers which will allow the handling of a higher sample volume. The same authors have evaluated the Lichrolut EN in a similar procedure. From 95 ml samples and using 200 mg SPE cartridges, the on-line SPE-SFC-DAD system allowed detection at the low 0.1 $\mu\text{g/l}$ in aqueous samples.

5.5. CONCLUSION AND FURTHER TRENDS

In recent years there has been an obvious development of on-line techniques in pesticide analysis, owing to the growing concern about the quality of our environment. When we look at that development, it is surprising to see that GC had been the preferred method of environmental chemists for a long time, but that on-line systems using LC became the first robust on-line technique. Since many recent pesticides and/or degradation products cannot be analyzed by GC, the only possibility of multiresidue analysis including compounds over a wide range of polarity is given by LC.

The on-line coupling of SPE with LC has become a routine method in many laboratories. Automation of the whole process (extraction and chromatography) is advantageously achieved by use of the Prospekt or OSP-2 which are also called "the second generation" of on-line systems. In these the coupling is extremely well done and avoids peak band broadening whilst giving good transfer of the analytes from the precolumn to the analytical column. Future research will certainly be devoted to the development of more specific packing materials, in order to avoid interference effects and reduce the limit-of-determination in real-life waters. There is also a need for developing sorbents or methods for highly polar and permanently charged compounds in order to widen the range of analyzable compounds.

Its wide acceptance, along with its identification potential, has contributed greatly to the development of SPE-LC-MS systems. This technique now appears to be well established, with the possibility of the coupling of LC-MS using a high flow, pneumatically assisted electrospray and atmospheric pressure chemical ionization interfaces which complement the use of thermospray in on-line SPE studies. The improvement in these new interfacing systems for pesticide analysis has been clearly demonstrated and opens new prospects for the confirmation of pesticides in natural water by reducing the LODs and by providing additional structural information; these are clear advantages over thermospray.

The on-line coupling of SPE-GC is a promising tool. The problem presented by water will certainly be solved either by the development of water-resistant retention gaps, or by more efficient on-line removal of water. Its development depends on the availability of interfaces between the aqueous part of the SPE and the non-aqueous

GC part. Although some studies have demonstrated the versatility and robustness of the technique, effort is still required to make it more accepted. As far as SFE is concerned, on-line developments are still needed, including on-line derivatization, SPE, clean-up, and on-line coupling with GC, LC or SFC.

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Immunochemical Methods and Biosensors

6.1. INTRODUCTION

An increasing effort is now being put into the environmental monitoring of compounds that may pose a risk to human and ecosystem health. Monitoring is also required for a better knowledge of the fate and transport of pesticides in the environment. The time and expense involved in classical analytical methods (i.e., sampling, sample preparation, and laboratory analysis) limit the number of samples that can be analyzed in environmental surveys. This stresses the need for developing fast, easy to use, robust, sensitive, cost-effective and field-analytical techniques.

Immunochemical methods were first developed for the purposes outlined above. They are based on the antigen–antibody interaction which is characterized both by high affinity and specificity. These two features make possible good analytical tools for sensitive and selective determinations. The first immunoassay was developed for the determination of insulin in blood by Yalow and Berson in 1960. The Nobel Prize was awarded in 1977 for this work [1]. Since that time, immunoassays have been commonly used in clinical chemistry for the detection of a wide range of compounds such as hormones, drug and viruses. They are very simple to run, one example being the use of the test kits for pregnancy hormones which have become home tests. However, the development of immunochemical methods in environmental analysis is relatively recent. The first papers only appeared in the early 1970s [2]. Although Hammock and Mumma recognized in 1980 the possibility of adapting immunoassays and using immunochemistry for environmental analysis [3,4], few immunoassays (IAs) were available commercially for pesticides in Europe in the early 1990s [5]. However, in recent years the use of IAs for environmental analysis has increased dramatically, and many examples in small- and large-scale studies have demonstrated the reliability of this technique, its complementary nature and advantages over conventional analytical methods. Information is now available on data interpretation, on quality assurance, and on quality control to improve the reliability of IAs. This trend is shown by their increasing acceptance by regulatory agencies, and a few immunoassays have re-

cently been accepted as screening methods by the US EPA and for the total determination of triazines. Current research includes the development of new assay formats, multi-residue assays, and flow-injection immunoassays. IAs are now also being coupled to other technologies.

Immunochemical methods involve the use of antibodies, which are polypeptide molecules produced by the immune-system cells when they are exposed to an antigenic substance. Antibodies are the key components of all immunochemical methods and are characterized by specific recognition sites in their structures which enable highly specific interactions with the antigens. The generation of antibodies for small molecules such as those of pesticides and many environmental contaminants is difficult because small molecules are unable to produce an immune response. This is certainly the major reason for the later introduction of immunochemical methods into environmental analysis than the clinical field. On the other hand, recent progress in the strategies for the rational design of haptens for the development of antibodies to small molecules partly explains the strong increase observed in recent years.

Immunochemical methods in pesticide analysis include immunoassays, the use of antibodies for sample preparation, i.e., for solid-phase extraction and the clean-up of samples, for detection in flow injection analysis, and for biosensors. The first immunochemical methods intensively developed for pesticides analysis were the immunoassays. Most of the commercial IAs involve a competitive reaction between antigen molecules, generally of the targeted pesticide, and labelled antigen molecules for a limited number of antibodies. The labels capable of detecting the immunological reaction with the purpose of giving quantitative measurements can be fluorescent, chemiluminescent, enzymes, or radioisotopes. In the early development of IAs for environmental analysis, radioimmunoassays (RIAs) were well implemented in clinical diagnostics because of their higher sensitivity relative to enzyme immunoassays (EIAs). Mumma has greatly contributed to demonstrating that EIAs can also be very sensitive and are better adapted to environmental analysis, so the EIAs are now those most commonly available in pesticide analysis. Especially important are the enzyme-linked immunosorbent assays (ELISAs), in which antibodies (or antigens) are immobilized on a solid phase to facilitate the separation of free and bound fractions.

ELISAs have now become popular for the rapid screening of organic pollutants in the environment, as is shown in numerous reviews [2–18]. Field-portable ELISAs permits the rapid on-site determination of pollutants for environmental control or for the selection of samples to be analyzed further in the laboratory. However, even if the method appears simple, an understanding of its basic principles is required as well as a presentation of the specific terms inherent to the technique. Some knowledge of the fundamentals of the method is necessary in making a good use of an IA, and especially in understanding why some IAs can be highly selective and sensitive for some target pesticides, whereas other cannot. Special attention is given to the

cross-reactivity processes, to the effect of the sample matrix, to data interpretation, and to validation procedures.

Sample preparation based on immunochemical methods is also an increasing area of research. The development of immunosorbents which can trap a class of pesticides is briefly described.

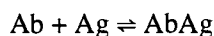
Whilst the IAs have become popular instruments for pesticide analysis, antibodies have also found application in biosensors. A biosensor combines a biological sensing element (using, e.g., enzymes, antibodies or cell receptors) which is connected to a transducer (e.g., an electrochemical, optical or piezoelectric device). Following an interaction between one target analyte and the biological component, some properties of the transducer are modified and give rise to a measurement. The development of biosensors is now an active area of research, and advances in the field of pesticide monitoring are presented in the last part of this chapter.

6.2. IMMUNOASSAYS

Immunoassay techniques were first developed in clinical chemistry to detect and determine organic compounds at low concentrations – down to the femtogram level (10^{-15} g) in some instances. This high sensitivity primarily results from the characteristics of the chemical reaction involved in the process.

6.2.1. The antigen-antibody interaction

Immunoassays provide an analytical technique for measuring the concentration of analytes, usually called antigens (Ag) or haptens. The basic principle of an IA depends on the antigen-antibody reaction:



which follows the law of mass action, with $K = [\text{AbAg}]/[\text{Ab}][\text{Ag}]$. The K values are often in the range 10^9 – 10^{12} l/mol and are high in comparison to the K values of biological or mineral complexes involving other types of interactions. This strong affinity explains the fact that the complex can be formed with very low concentrations of antigen or antibodies.

In most IAs, the antibodies are immobilized on a solid support and a measurement of the binding sites by the analytes is made because the antibody occupancy reflects the concentration of analytes in the medium. However, since the binding reaction does not produce a signal, a tracer should be added which allows one to estimate the Ab occupancy by measuring the tracer signal. IAs are classified according to a number of criteria, the most important being whether occupied or unoccupied binding sites are measured.

6.2.2. Description of immunoassays

6.2.2.1. Competitive and non-competitive immunoassays

Competitive IAs involve the measurement of unoccupied sites when using a limiting Ab concentration. Figure 6.1 illustrates immunoassays based on heterogeneous conditions with either antibodies (Fig. 6.1a) or antigens (Fig. 6.1b) immobilized. In Fig. 6.1a, the sample analytes and labelled analytes compete for antibody binding sites on the support. After a separation method which allows one to remove free analytes and free labelled analytes from the medium, the concentration of the bound labelled antigens is measured by the signal tracer. In indirect competitive ELISAs, the antigens are immobilized on the solid support and labelled antibodies are added to the medium together with the analytes to be determined. In Fig. 6.1b, immobilized analytes compete with the sample analytes for non-labelled antibodies. The concentration of antibodies linked to the immobilized analytes is determined by the addition of labelled antibodies which can specifically recognize antibodies.

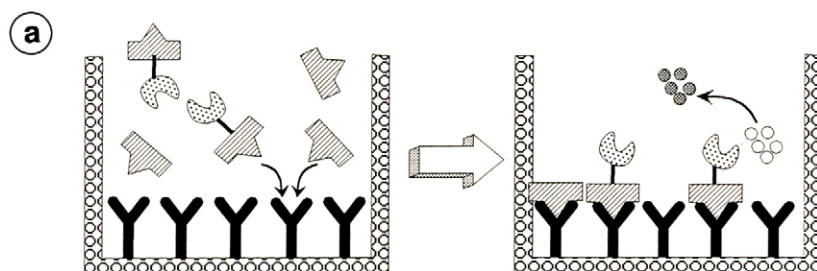
Non-competitive IAs are shown in Fig 6.1c and rely on the measurement of the occupied binding sites by using excess concentrations of Abs. Unlabelled antibodies are immobilized whilst labelled antibodies are added to the medium. This configuration can be performed only when the analyte of interest possesses at least two binding sites, and is therefore not appropriate with small pesticide molecules.

Most of the commercial IAs use the competitive configuration indicated in Fig. 6.1a. The tracer consists of a labelled analyte which is added to the system. If the Ab and the tracer concentration are kept constant in an assay, and if there is a limited number of binding sites, the proportion of bound tracers decreases with an increasing analyte concentration. In radiochemically labelled IAs (RIAs), the tracer is the radiolabelled analyte itself. Although fluorescent and chemiluminescent labels have gained in popularity in the past few years, enzyme labels such as horseradish peroxidase or alkaline phosphatase are still the most common. The labelled antigen is often named the “enzyme conjugate” in an ELISA.

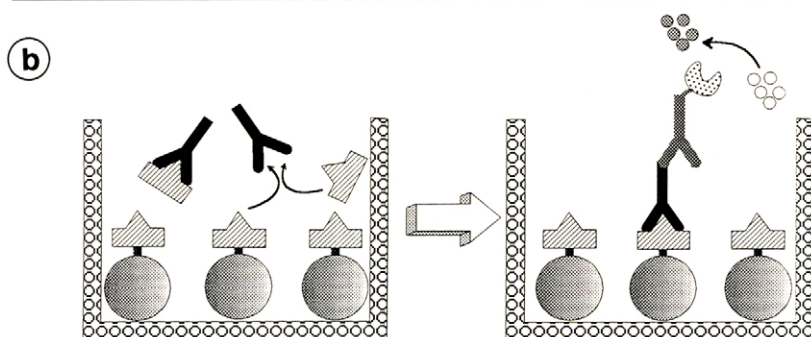
6.2.2.2. Description of an ELISA sequence

Enzyme-linked immunosorbent assays are heterogeneous assays because the Abs or Ags are immobilized on a solid phase. Figure 6.2 summarizes a typical sequence, as involved in most ELISAs. Two formats are commercially available and differ only in the solid surface used for the immobilization. One common format starts the sequence with antibody-coated tubes or wells (i.e., Millipore, ImmunoSystem, Ensys, Quantix and most laboratory-made IAs). Another format consists of antibodies covalently bound to 1 μm magnetic particles which act as the solid support (Ohmicron systems). In the tube or well format the sample (typical volumes 100–200 μl) and a known amount of labelled antigen are added and compete for the limited number of binding sites in the coated tube or well. In the magnetic-particle format, the sample (typical volume 250 μl), a known amount of enzyme conjugate, and

Direct Competitive ELISA



Indirect Competitive ELISA



Sandwich ELISA

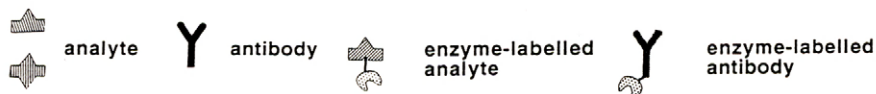
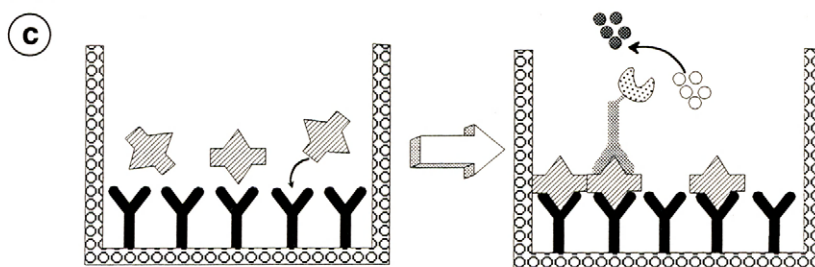


Fig. 6.1. Principles of immunoassays (a) Competitive IA with immobilized antibodies (limiting antibody concentration) and addition of both the analytes (or antigens) and labelled analytes to the solution; (b) competitive IA with immobilized antigens and addition of both analytes and antibodies (limiting antibody concentration) to the solution; (c) non-competitive IA with unlabelled immobilized antibodies and addition of both the analyte and an excess of labelled antibody. From Ref. [6].

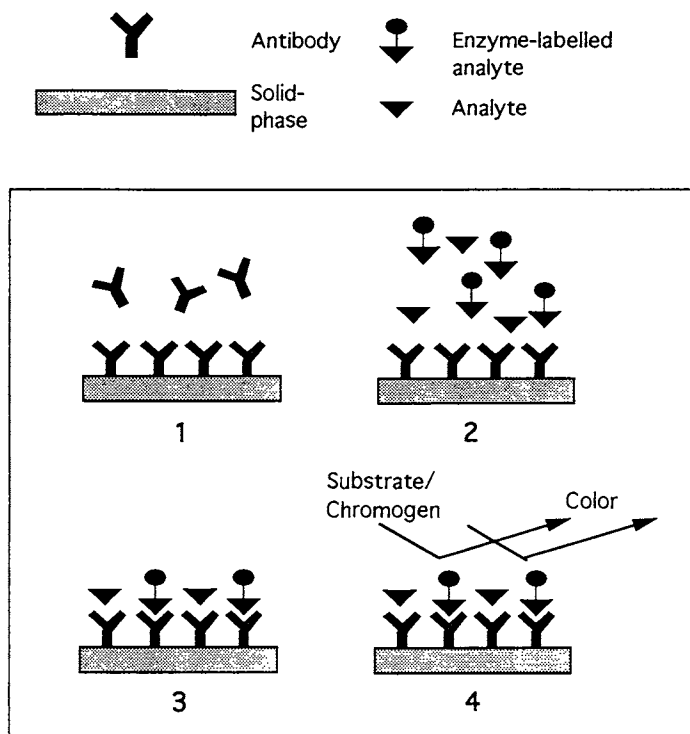


Fig. 6.2. Sequence of an ELISA test. (1) Immobilization of a known amount of antibodies to a solid-phase; (2) competition between the pesticide molecules in the sample and a known amount of enzyme conjugate molecules for a limited number of binding sites; (3) after incubation, separation of the bound enzyme conjugates and/or pesticides from the free ones; (4) addition of the substrate and chromogen, incubation during a certain time, and addition of a stopping reagent.

a known amount of particles are added in a test tube. Then, in both formats, there is an incubation step at room temperature during 10–60 min, depending on the assay. According to the law of mass action, the more analyte there is in the sample, the more enzyme conjugate it will displace from the binding sites. The original analyte concentration in the sample can be determined by measuring the amount of enzyme conjugate bound to the antibodies. However, one can quantitate, provided that unbound (or free) conjugates and antigens have been removed from the solution. When antibodies have been coated to the wall of the test tube this step is simply performed by washing the tube. When using magnetic particles, the test tube is placed in a magnetic field which pulls the magnetic particles to the bottom of the test tube and washing allows the separation of bound analytes and conjugates from unbound ones. Then, the amount of enzyme conjugate bound to the antibodies is measured by adding a substrate and a chromogen to detect the enzyme. The enzyme reacts with the substrate, which in turn causes the chromogen to produce a coloured

compound. After incubation, the reaction is stopped by performing a “stopping reaction”.

The colour produced is proportional to the amount of bound enzyme conjugate and therefore inversely proportional to the amount of analytes present in the sample. A darker colour means less analytes in the sample whereas a light colour indicates a high concentration of analytes in the sample. Quantitative measurements can be obtained by running standard samples having known concentrations and by measuring the absorbance at the maximal absorption wavelength using a spectrophotometer.

6.2.3. Antibodies

Antibodies are the critical part of all IAs, since their quality greatly contributes to the sensitivity and specificity. However, besides their specificity, antibodies are also known for their cross-reactivity, i.e., the extent to which they react with compounds structurally related to the analyte, or sometimes with entirely different compounds. The degree of cross-reactivity depends on both the pesticide molecule and on the way in which the antibodies are created. As the target molecule becomes smaller, the number of determinant groups in the molecules decreases and the probability of cross-reactivity increases. Moreover, if the target pesticide belongs to a class containing many other compounds of similar structure and/or if the target pesticide is degraded or metabolized in structurally close compounds, then cross-reactivity can be expected and much care will have to be taken in order to avoid it as much as possible for a very specific IA towards the single target pesticide. On another hand, if the objective is to obtain a class-specific IA for screening purpose, then the cross-reactivity can be emphasized at maximum.

6.2.3.1. General scheme for antibody production

The technology for developing specific antibodies is an active area, sometimes using very sophisticated methods. One has to realize, however, that up to now the commercial IAs use mainly polyclonal and monoclonal antibodies. The route for producing polyclonal and monoclonal antibodies follows a similar scheme up to the immunization. Because compounds of low molecular mass (<1000), such as most of the pesticide molecules, are unable to evoke an immune response, before immunization they have to be modified by binding to a larger carrier, usually a protein. Therefore, the generation of Abs become more complex, and involve the steps described below:

- selection of the target pesticide molecule;
- design of a hapten, which consists of the synthesis of a derivative of the target molecules so that it will contain an appropriate group for attachment to a protein;
- covalent binding of the hapten to a carrier protein, to form an immunoconjugate; and
- animal immunization.

have been determined in the target molecule, the handle should be attached as far as possible from these sites, because their exposure of the molecule to the immune system will then be maximized. The smaller the molecule is, the more important the contribution of each group. Nevertheless, the strategy will be different depending on whether a single compound within a class is targeted or the whole class. For the class-selective assay, the handle will be best located at a position which leaves the common sites exposed to the immune system. This is illustrated in Fig. 6.4a. If the hapten is made by modifying the atrazine molecule through the amino group in position 1, then it is obvious that the antibody will be unable to distinguish between atrazine and propazine and, as shown later, a certain degree of cross-reactivity is obtained with other chlorotriazines. Using the other amino group, in position 2, will also lead to Abs showing some degree of cross-reactivity towards other triazines, especially simazine. If the handle is attached via the chloro group in position 3, then we can expect cross-reactivity with prometryn. The structure of the compounds within the chlorotriazine group explains the difficulty of obtaining a specific Ab for atrazine. The effect of the Abs prepared from these different haptens for atrazine upon the properties of the corresponding IAs is examined later.

One is also recommended to retain the identity of all determinant groups in small molecules, and therefore to avoid using analyte functional groups, although the chemical modification should be easier. The example most often cited is the easy modification of an amino group, by the preparation of the hemisuccinate of the hapten, because the characteristics of the amino group, i.e., its basic nature and moderate H-bonding donor/acceptor properties, are modified [19]. Derivatization through the carbon atoms of the target molecules is preferred since these atoms do not contribute as much as heteroatoms to the steric and electronic properties of the molecule. One example is shown for the bromacil molecule, for which Abs obtained by attachment of the carrier protein through the methyl group in position 2 gave better IAs than Abs obtained by modifying the amino group in position 1 (Fig. 6.4b) [23]. Another example, regarding the preparation of Abs for chloroacetanilide herbicides is represented in Fig. 6.4c. Here, the easiest way is to use one of the two functional groups in position 1 or 2, although the best site to avoid cross-reactivity within the class should be at position 3. Most studies have reported the modification of alachlor at position 1 or 2, with the corresponding Abs having little cross-reactivity with compounds within the group, thus showing that characteristic portions of the molecules are sometimes sufficient to generate valuable Abs [24–26]. However, some cross-reactivity was observed with one metabolite. Modification in position 3 led to Abs capable of recognizing alachlor significantly better than the other compounds within the class [27]. Phenylurea herbicides have also received much attention and for this group, as shown in Fig. 6.4d, attachment to the carrier protein through the 1-methyl group provides the easiest means of respecting the identity of the molecule. However, because of the structure of this group of compounds, such Abs are not totally

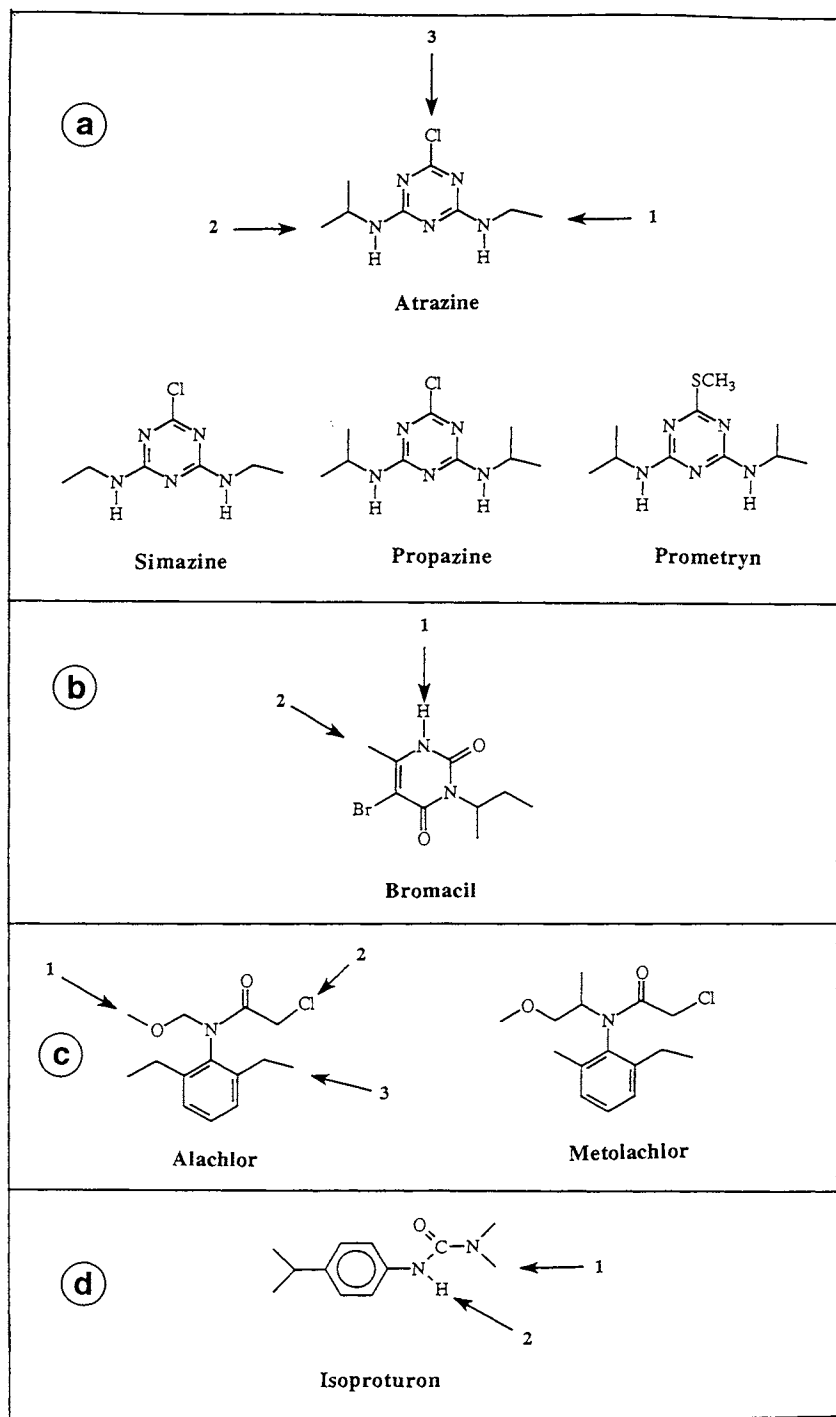


Fig. 6.4. Structure of some pesticides and corresponding positions for linker location.

selective. Although site 2 has also been considered, the more sensitive assays were obtained by modification at position 1 [28–30].

With small molecules, the use of a spacer-arm in the linker is required in order to favour recognition by the immune system. This spacer helps in holding the target-analyte structure away from the protein. The length and the chemical structure of the linker should be selected in order to maximize the exposure of the target molecules, and the spacer itself should not be selectively recognized. First, the use of a functional group in the chemical structure should be avoided because this could alter the electronic distribution of the target molecule. It has been reported that aminotriazole haptens, prepared by using the hetero-bifunctional reagent maleimidobenzoic acid *N*-hydrosuccinimide, produced Abs that recognized the hapten protein conjugate, but not the target analyte [18,31]. The length for the spacer of three to six atoms has been shown to be optimal.

Computer modelling will certainly play an increasing role in the design of the haptens. This step is really the most important one for the Ab specificity and sensitivity. It can be laborious and time-consuming, involving two or three intermediate syntheses. Whenever possible, commercially available starting products or known degradation products should be used.

6.2.3.1.3. Immunoconjugate. Once the hapten has been synthesized, it should be linked to an appropriate protein. Several methods can be used and have been reviewed [18,32,33]. The selection of the conjugation method depends mainly on the functional group of the hapten. One commonly encountered method for haptens containing a carboxylic acid group uses the carbodiimide/*N*-hydroxysuccinimide procedure which transforms the carboxylic group into an activated ester, thus allowing a covalent link to be formed via an amino group of the protein. The stability of the hapten during this reaction is important and some functional group protection may be required prior to protein conjugation. The carrier proteins most frequently used in IAs are keyhole limpet hemocyn (KLH), tyroglobulin (TG), conalbumin (CONA), bovine serum albumin (BSA), bovine β -lactaglobulin (β -LG) or ovalbumin (OVA).

It is worthwhile verifying the coupling reaction and characterizing the immunoconjugate structure, before immunization, by the number of haptens attached to the carrier protein (named the epitopic density of the hapten-protein conjugate). From literature data, it is evident that about 10–30 haptenic groups per 100 kDa of carrier protein are required to obtain good antibody production [34]. When using BSA or β -LG as a carrier protein, the epitopic density measurement is usually performed using spectrophotometric measurements of immunoconjugates and of conjugates modified with 2,4,6-trinitrobenzenesulfonic acid (TNBS) reagent. The TNBS reacts specifically with the primary amino groups in proteins and is widely used for the chemical modification and quantification of lysine residues [35–37]. The specific reaction of TNBS when applied to the immunoconjugate allows one to determine the free lysine

side chains and then to deduce the number of bound hapten molecules per protein carrier. Other means for determining the epitopic density include the use of labelled haptens and are based on spectrophotometric and electrophoretic methods [32,33, 36,38]. Matrix-assisted laser desorption mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS) have also been employed [37,39, 40]. The ESI-MS methods make it possible to determine the number of herbicide molecules linked to the protein by comparing the average molecular weight of the hapten–protein conjugate with that of the natural protein. The method was applied to the characterization of β -LG immunoconjugates prepared from atrazine and isoproturon and the results were in agreement with those obtained from the TNBS method [37]. However, the characterization by MS of larger protein conjugates requires further investigation.

6.2.3.2. *Antibody structure*

Antibodies belong to the class of immunoglobulins-G (IgG). An IgG molecule consists of two identical heavy chains, each of 450 amino acid residues, and of two light chains, each of 212 amino acid residues [41]. The two heavy chains are held together by disulfide (–S–S–) bonds and one heavy chain is connected to one light chain by a similar bond. The four chains are shaped like the letter Y. Both the heavy chain (see Fig. 6.5) and light chains are divided into constant and variable domains based on their amino acid sequence variability. The constant region corresponds to 90% of the total mass of the antibody. In the variable region, there is considerable amino acid variability from one antibody to another. The most important regions of an antibody with regard to the antibody–antigen interaction are in the variable region. The interaction is caused by the complementary spatial distribution between the antigen and the antibody. The formation of hydrogen bonds, electrostatic interactions, van der Waal's forces, and hydrophobic interactions, helps in the strong affinity between the antibody and the antigen. Investigations are now under way to give better knowledge of the characteristics of antibodies. Micellar electrokinetic capillary chromatography and MALDI-MS have been used to monitor the thermal stability of antibodies [42].

6.2.3.3. *Polyclonal antibodies*

The production of polyclonal antibodies (pAbs) is performed by first immunizing animals. Although any animal can be used, pAbs are often produced in rabbits because they are easy to care for and moderate amounts of serum are easily obtained. Much larger amounts of pAbs can be obtained using sheep, goats or cows.

In the immune system, a single B-lymphocyte produces a single type of Ab molecule. In a typical response to an immunoconjugate, B-lymphocytes produce several different Ab molecules all directed to different parts of the immunoconjugate. An antiserum for a particular immunoconjugate is, in fact, a mixture of Abs produced by various clones of antibody-producing cells, so that polyclonal Abs are heterogeneous with various affinities and different analyte recognition.

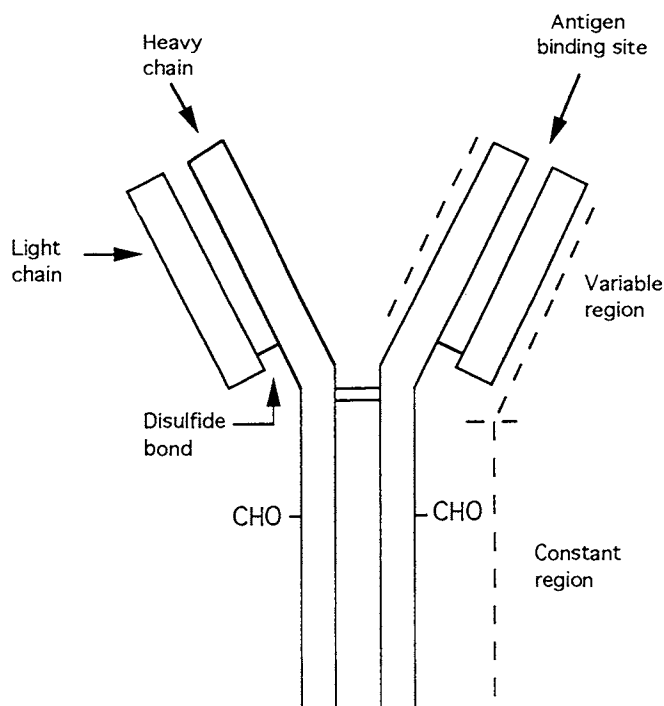


Fig. 6.5. Basic structure of an IgG antibody molecule.

The quality of the Abs depends to a certain extent on the animal's immune system, the immunoconjugate, and the immunization schedule. It is recommended that one should immunize at least two or three rabbits in order to minimize the animal variability. Common protocols involve a first intradermal injection of the immunoconjugate mixed with an adjuvant to enhance the immune response, and then a series of secondary injections, so-called "boosters", every 4 or 5 weeks. After each boost, serum samples are collected and the Ab titre is measured, which allows one to check the progress of the immunization. After a period, usually of 3–6 months, the Ab titre no longer increases. The collected serum has to be purified first by isolation of the IgG fraction, usually performed by affinity chromatography.

The first period of assay development involved almost exclusively polyclonal Abs. These have shown to provide good IAs for both research and commercial purposes at low cost, so that polyclonal Abs are unlikely to be replaced in the near future. They do have some limitations because polyclonal antiserum can vary from one animal to another and the supply of Abs ends when the animal dies. These problems are solved commercially by using serum pools that are carefully characterized. However, a consistent source of Ab product cannot be guaranteed and it is increasingly attractive to have a constant supply of identical antisera. This can be provided by the hybridoma technique which generates homogeneous monoclonal antibodies.

6.2.3.4. Monoclonal antibodies

The technique for the production of monoclonal (homogeneous) antibodies (mAbs) was first developed in 1975 by Köhler and Milstein [43] and was adopted for pesticide IAs around 1985. The B-lymphocytes are able to produce antibodies but are unable to grow in culture. The hybridoma technology involves their immortalization by fusion with tumorigenic B-lymphocytes as myeloma cells. The production of monoclonal Abs is well documented in the literature and involves the steps shown in Fig. 6.6 [4,41–46]. The first step is the immunization of the mouse with the appropriate immunoconjugate to provoke its immune system. The mouse spleen is removed and the spleen cells or splenocytes are fused with myelomas with polyethylene glycol (PEG). The resulting hybridomas possess both the antibody-producing ability and the immortality of the parent cells. Unfused splenocytes die after a short period of growth and unfused myelomas are removed using a medium containing hypoxanthine, aminopterin and thymidine (HAT). This permits the selection of a population of fused hybridoma cells which are then tested for their ability to produce the desired Ab, normally using a competitive enzyme immunoassay or super immuno-paramagnetic beads [45]. The fusion mixture is then divided into many culture

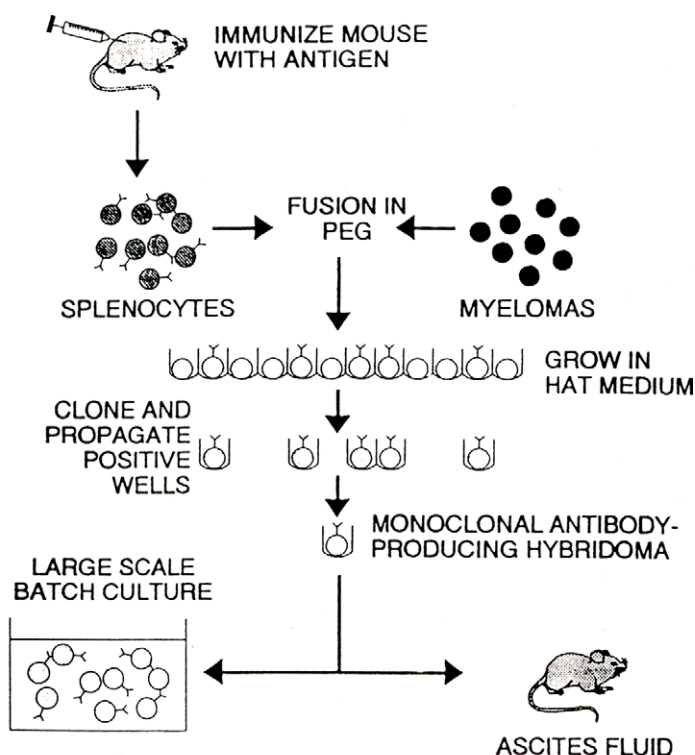


Fig. 6.6. Production of monoclonal antibodies from the mouse. From Ref. [41].

wells and allowed to grow. The presence of Ab is tested again and the positive wells are further cloned. Suitable clones are propagated on a larger scale in culture flasks or in larger bioreactors. They can also be made *in vivo* via ascetic fluids in mice, but with low yields. One critical step in the mAb production is the development of an efficient screening procedure to select cell lines secreting high quality Abs [4].

The advantage of this technology is that it provides an unlimited production of mAbs with constant affinities and cross-reactivities. However, in contrast to pAb production, the hybridoma technique is more difficult, laborious, time-consuming and expensive. However, many laboratories now have the capability to prepare both pAbs and mAbs and an increasing proportion of IAs using mAbs is being reported in the literature or introduced in commercial kits. One advantage of using mAbs in commercial kits is that they provide a long-term supply of kits with well characterized and constant performance. However, one must not forget that the choice of the degree of selectivity and specificity depends on the further and intended applications of the antibodies, and is different for IAs, immunoaffinity columns, or biosensors. As each cell is cultured separately during the production, mAbs show less cross-reactivity than pAbs and in general, for very specific measurement, mAb should be preferred. However, in some cases cross-reactivity cannot be avoided, depending on the hapten design. For example, mAb derived from the hapten of atrazine modified via the amino group (position 1 in Fig 6.4a) will show high cross-reactivity with propazine.

6.2.3.5. *New technologies for antibody production*

Techniques for cloning and engineering antibodies are evolving rapidly. Recombinant DNA technology is a very active area even if, at present, the development of recombinant antibodies is very expensive and time-consuming [47]. Molecular biology techniques now allow the amplification, selection and alteration of antibodies expressed in *Escherichia coli*. There is an interest in recombinant antibodies in environmental analysis, to speed up the development of new Abs, and to become independent of animal experiments.

Each antibody molecule has two binding sites for the same antigen. Chemical, biological and genetic methods have been developed for the production of bifunctional antibodies which recognize a different antigen at each of the two binding sites and these antibodies have found interesting applications in the medical field [42,48, 49]. The constant region of the antibody has been shown to have little effect in many applications, and different methods can be used to remove this fragment of the antibodies, in order to isolate smaller fragments containing the binding sites. Enzymatic degradation with pepsin leaves the two portions of the antibodies still connected, with the two binding sites, whereas papain cuts out the antibody into two separate fragments each containing a binding site. Other fragmentation has been achieved by genetic methods which have now provided the possibility of producing desired antibodies or fragments from bacterial yeast or cell cultures. It is possible to construct a

gene library, and combinations of gene and/or fragments, for producing tailor-made antibodies with the desired affinities.

The molecular genetic technique known as combinatorial phage has found some applications in environmental analysis [45,50,51]. Genes of the fragmented antibodies can be isolated from cells of the immune system of the animal and then be combined and expressed as antibody molecules on the surface of a phage particle (a virus that infects bacterial cells). Once the antibodies with the required affinity have been selected, they can easily be produced by bacterial cells. Antibodies against *S*-triazines have been developed using such a phage system [45]. In another study, the antibody genes were sequenced from hybridomas secreting monoclonal antibodies with different cross-reactivity patterns against atrazine and terbutryn [52]. This allowed a better understanding of the antigen recognition and of the cross-reactivity patterns of Abs to different pesticide residues. Identification of the contact residues responsible for cross-reactivity allows one to alter the Ab structure genetically, so single-analyte or class-specific Abs should be optimized in the future. With a similar approach, the recombinant antibody technology and computational modelling have made it possible to deduce a model for the phenylurea combining sites [53].

6.2.4. Characteristics of competitive immunoassays (ELISAs)

Immunoassays, whatever their format, contain a target analyte or antigen, a specific antibody which binds to the analyte, and a corresponding hapten conjugated to a protein/enzyme. There is no doubt that the performances of immunoassays are primarily a function of the affinity and selectivity of the antibody. However, the careful design of the labelled-analyte conjugate competitor has been shown to be important for the sensitivity of ELISA, although the requirements in the chemical structure of this analyte derivative are not so strict as for the immunizing hapten.

6.2.4.1. Competition reaction with enzyme-labelled haptens

Both the enzyme conjugates and the analytes in the unknown sample compete for a limited number of binding sites, according to a reversible antibody-analyte equilibrium competition with an antibody-enzyme conjugate as represented in Fig. 6.7. If the affinity of the antibody for the competitor is lower than that for the target analytes, then small concentrations of the analytes will shift the equilibrium conditions to favour formation of the antibody-analyte complex. This will inhibit the association of the antibody with the labelled competitor [22]. Consequently, the structure of the labelled competitor has a strong influence on the sensitivity of the assay, since it affects the equilibrium conditions. Usually, the structure of the hapten competitor is slightly different from that of the immunizing hapten, by varying in the attachment site, chain length, and functional group of the spacer arm [18,22,27–29,54–56]. As with the immunizing haptens, guidelines have been given for optimizing the design of the competitor conjugate, taking into account the cross-reactivity of the haptens [19,29,57].

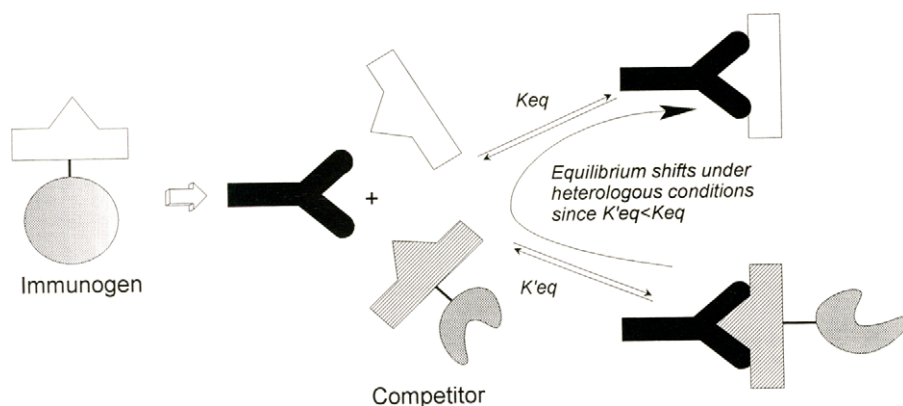


Fig. 6.7. Scheme for representing the shift of the equilibrium in competitive immunoassays. The affinity of the antibody for the competitor should be lower than that for the analyte. From Ref. [18].

6.2.4.2. Dose-response curve

In an ELISA format with a known amount of antibodies linked to a solid support and a fixed concentration of enzyme conjugates, the photometric determination of the enzyme activity by absorption is related to the analyte concentration via a dose-response curve such as that represented in Fig. 6.8. Such calibration curves are constructed with standard antigen concentrations and have a sigmoidal shape with a linear portion. When the analyte concentration is very low, the equilibrium is in favour of a high amount of enzyme conjugate linked to the antibodies, and the corresponding absorbance is maximal. The working range of the calibration curve is defined by the lower and upper limits that can be exploited. Within this range, the change in absorbance correlates with the analyte concentration. At a higher concentration than the upper limit, the assay is saturated, and an increase in the analyte concentration no longer has an effect. Many experimental dose-response curves can be found in the literature, or are provided with commercial kits. The working range of the dose-response is an important feature which gives a first indication of the sensitivity of the test.

The most common representation gives the variation of the absorbance, A , using a logarithmic abscissa for the concentration. However, to allow direct comparison of several standard curves, the absorbance data are normalized between 100%, which corresponds to the absorption of a zero control (A_0), and 0%, which corresponds to the absorbance of a standard excess (A_{excess}). The transformation is performed according to $\%B/B_0$ values according to the formula

$$\%B/B_0 = 100(A - A_{\text{excess}})/(A_0 - A_{\text{excess}})$$

where A is the absorbance of the sample or standard. The standard curves, obtained

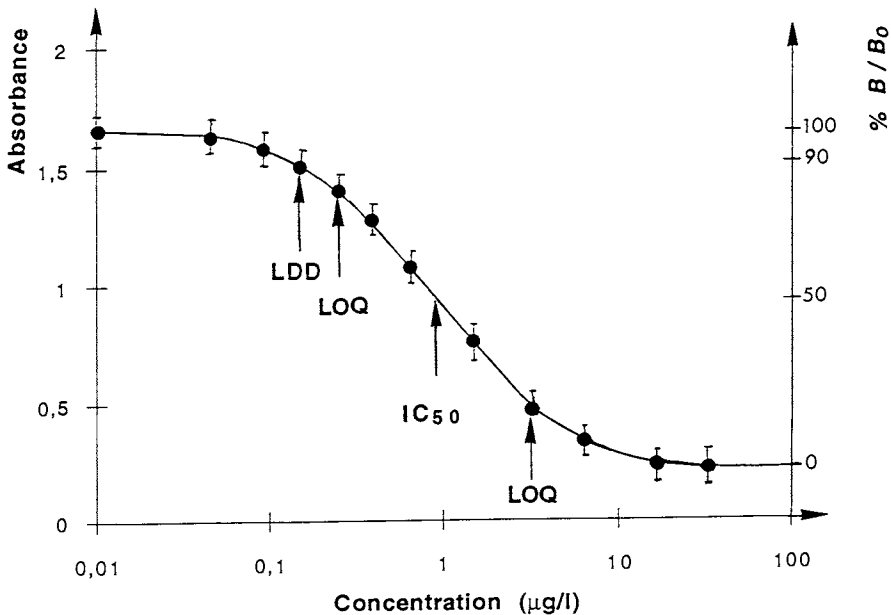


Fig. 6.8. Typical sigmoidal dose response as measured by the absorbance of the solution after incubation with a fixed concentration of antibodies and enzyme conjugates, with increasing concentration of antigen. Definition of usual parameters: limit of detection or least detectable dose (LDD), lower and upper limit of quantification (LOQ), inhibition concentration at 50% (IC_{50}). A_0 and A_{excess} are the absorbances of the zero control solution and of the standard excess solution.

by reporting A or $\%B/B_0$ as a function of the logarithm of the analyte concentration, are convenient to work with because the raw data can easily be reported on a graph, especially when a standard spectrophotometer is used. Various mathematical transformations have been proposed for a better linearization of the standard curves [58,59]. Companies which sell IAs provide microprocessors which automatically convert immunoassay optical readings to sample concentration, and the transformations are given in the commercial characteristics of the kit. One representation often selected for the microprocessors has the form logit versus $\log c$. The most used transform concerns the normalized response, and is defined by the following equation:

$$\text{logit } B/B_0 = \log(B/B_0)/(1 - B/B_0)$$

This logit transform attempts to add the tailed regions of the sigmoidal curve into the linear transform by reporting $\text{logit } B/B_0$ as a function of the logarithmic concentration of the analyte. However, one has to take note that the scale on the y axis is no longer linear, since the logit transform compresses the response in the middle of the curve and expands the low and high ends of the curve [60,61].

Usually, the dose-response curve is calibrated only within the working range. In most of the ELISAs for pesticides, this range is obtained for about two log units. Table 6.1 reports the main characteristics of the commercially available ELISAs for pesticides, including the measuring range. Table 6.2 lists the immunoassays described in the literature, most of them being developed in laboratories. One can see that there has been great development of many IAs in recent years, but not all of them have become commercially available. For the majority of the commercial and laboratory ELISAs, the lower limits of the working range are at concentration levels 0.02–0.1 $\mu\text{g/l}$ and the upper limits at 5–10 $\mu\text{g/l}$. They therefore easily meet the EU requirements for drinking-water regulatory control without requiring a concentration step. One example of a linear curve is given in Fig. 6.9a for the determination of standard dose-response curves using a commercial kit for alachlor. For this, a linear range was verified for concentrations varying from 0.1 to 5 $\mu\text{g/l}$ [62]. In Fig 6.9b, there is one example of a logit B/B_0 transform, demonstrating the non-linearity of the y ordinate in the experimental calibration curve of an ELISA kit for pentachlorophenol [61].

6.2.4.3. Sensitivity: limit of detection and limit of quantification

The sensitivity of an IA is usually expressed either as the limit of detection or as the smallest concentration of the analyte that produces a signal which can be significantly distinguished from zero for a given sample matrix with a stated degree of confidence. The minimum detectable concentration is often known as the “least detectable dose” (LDD). Although there is no standardized way of defining the sensitivity, there is a general consensus in favour of selecting the dose which inhibits 10% of the binding of the antibody with the enzyme tracer at 90% B/B_0 . The LDD is then often measured using the standard samples in pure water or buffer. The minimal detectable dose has been also described as being two or three times the standard deviation from the mean measurement of the blank dose signal. As can be seen in Table 6.1, the most sensitive assay is obtained for diazinon, with a LDD of 2 ng/l, and several assays have LDD below 50 ng/l.

The limit of quantification (LOQ) is more difficult to define and represents the level above which quantitative results can be obtained with a stated relative precision, or specified degree of confidence, in real samples [59]. In some cases, the LDD value at 90% B/B_0 is not in the linear range of the transformed dose-response curve, and the selected LOQ is defined by the upper limits of the linear range of the dose-response curve (see Fig. 6.8).

When the LDD is above the required value, it is still possible to apply a concentration step to the water sample using a solid-phase cartridge. However, one has to take account of the organic content of the concentrated sample. Solvent tolerance depends on the antibodies involved in the assay and on the organic solvent. Common organic solvents can be used in small amounts, i.e., ethanol, dioxane, dimethyl sulfoxide, propylene glycol, acetonitrile, tetrahydrofuran, or methanol [18]. Solvent-

TABLE 6.1
COMMERCIAL IMMUNOASSAYS (ELISAS) FOR PESTICIDE ANALYSIS IN AQUEOUS MATRICES

Analyte	Format	Ab	Quantification range	LDD ($\mu\text{g/l}$)	IC_{50} ($\mu\text{g/l}$)	Main cross-reactants (LDD; IC_{50} , in $\mu\text{g/l}$)
2,4-D	Ab-coated test tubes	nd		1.6		
Alachlor	Ab-coated test tubes	nd	0.1	0.073	0.5	Alachlor sulfonic acid (0.1); metalaxyl (0.1); metolachlor (0.1)
Carbofuran	Ab-coated test tubes	nd		0.1		ncr
Metolachlor	Ab-coated test tubes	nd		0.25		No data
Paraquat	Ab-coated test tubes	nd		20		No data
Triazine	Ab-coated test tubes	nd		0.053		Propazine (0.001); terbutylazine (0.01); simetryn (0.037); prometon (0.044), prometryn (0.076); hydroxy-atrazine (0.12); simazine (0.32); DEA (0.4)
2,4-D	Ab-coated 96-well plate	nd	0.5–100	0.1	2.3	2,4-D alkyl esters
Acetanilide (acetochlor)	Ab-coated 96-well plate	nd	0.1–5	0.02	1.5	Acetochlor, alachlor, butachlor, furalax, metalaxyl, metolachlor
Aldicarb	Ab-coated 96-well plate	nd	1	0.4	5	Aldicarb sulfone (0.6; 6)
Alachlor	Ab-coated 96-well plate	nd	0.1–2.5	0.046	0.6	Alachlor sulfonic acid (0.4; 3.6)
Carbendazim/benomyl	Ab-coated 96-well plate	nd	0.4–10	0.1	2	Thiophanate (0.1; 1.2); thiophanate methyl (0.1; 1.3)
Chlorpyrifos	Ab-coated 96-well plate	nd	0.1–1	0.1	0.3	Chlorpyrifos methyl (0.02; 0.1); fenclorophos (0.02; 0.34)
Cyanazine	Ab-coated 96-well plate	nd	0.25–	0.14	0.73	ncr
Chlorsulfuron	Ab-coated 96-well plate	nd	0.04–0.8	0.04	0.22	ncr
Diazinon	Ab-coated 96-well plate	nd	0.03–0.5	0.002	0.1	Diazoxon (0.2; 0.9)
Imazapyr	Ab-coated 96-well plate	nd	0.3–30	0.3	3	Imazethapyr (0.6; 8)
Isoproturon	Ab-coated 96-well plate	nd	0.05–0.5	0.02	0.13	ncr
Metalaxyl	Ab-coated 96-well plate	nd	0.1–25	0.1	0.3	Fluralax (0.07; 0.3)
Metolachlor	Ab-coated 96-well plate	nd	0.1–2	0.1	0.53	ncr

References pp. 509–517	Metsulfuron	Ab-coated 96-well plate	nd	0.025–0.5	0.020	0.1	Thifensulfuron (0.03; 0.25); sulfuron methyl (0.026; 0.47); triasulfuron (0.13; 0.96)
	Paraquat	Ab-coated 96-well plate	nd	0.025–0.2	0.017	0.079	Diquat (0.03; 0.77)
	Parathion (methyl)	Ab-coated 96-well plate	nd	0.04–0.4	0.03	0.3	Parathion-ethyl (0.05; 0.7)
	Thiabendazole	Ab-coated 96-well plate	nd	0.25–4	0.25		No data
	Triasulfuron	Ab-coated 96-well plate	nd	0.05–1	0.04	0.36	Metsulfuron methyl (0.15; 7)
	Triazine	Ab-coated 96-well plate	nd	0.05–20	0.05	0.25	Ametryn (0.01; 0.16); propazine (0.01; 0.19) DEA (0.17; 12); prometon (0.01; 0.29); prometryn (0.015; 0.27); simetryn (0.03; 1.13); simazine (0.065; 1.47)
	Triazine (high sensitivity)	Ab-coated 96-well plate	nd	0.01–0.5	0.01	0.080	Prometon (0.008; 0.03); simetryn (0.003; 0.07) propazine (0.01; 0.1); DEA (0.05; 2); simazine (0.04; 2)
	Urea herbicides (Chlortoluron)	Ab-coated 96-well plate	nd	0.05–2	0.04	0.53	Neburon (0.027; 0.21); diuron (0.02; 0.29); chlorbromuron (0.03; 0.71); linuron (0.02; 0.48); monuron (0.07; 2.3)
	Alachlor	Ab-bounded magnetic particle	pAb	0.05–5	0.05	0.98	Alachlor ESA (0.03; 2.98); metolachlor (6.0; 78)
	Atrazine (high sensitivity)	Ab-bounded magnetic particle	pAb	0.015–1	0.015	0.22	Propazine (0.005; 0.091); DEA (0.027; 0.87)
	Atrazine	Ab-bounded magnetic particle	0.05	0.05–5	0.046	0.72	Propazine (0.033; 0.74); ametryn (0.053; 0.39); prometryn (0.054; 0.64); prometon (0.056; 2.22); DEA (0.062; 3.21); simazine (0.34; 4.9); terbutylazine (0.31; 15)
	Cyanazine	Ab-bounded magnetic particle	pAb	0.04–3	0.035	0.43	Terbutylazine (0.05; 12)
	2,4-D	Ab-bounded magnetic particle	pAb	0.7–50	0.7	15	2–4-D alkyl esters
	Metolachlor	Ab-bounded magnetic particle	pAb	0.05–5	0.05	0.85	Acetochlor (0.06; 6.55); metalaxyl (0.06; 5.6); butachlor (0.26; 52)
	Metribuzin	Ab-bounded magnetic particle					

TABLE 6.1 (CONTINUED)

Analyte	Format	Ab	Quantification range	LDD ($\mu\text{g/l}$)	IC_{50} ($\mu\text{g/l}$)	Main cross-reactants (LDD; IC_{50} , in $\mu\text{g/l}$)
Paraquat	Ab-bounded magnetic particle		0.20–0.5	0.020	0.30	Methylbipyridinyl methyl sulfonium salt (0.002; 0.96); diethyl paraquat (0.005; 13)
Silvex	Ab-bounded magnetic particle		5–250	1.4	58	Silvex isooctyl ester (0.25; 1.78); silvex methyl ester (0.04; 8.3); 2,4,5,-T (1.0; 77)
Aldicarb	Ab-bounded magnetic particle	pAb	0.25–100	0.25	9.28	Aldicarb sulfone (0.27; 193); Aldicarb sulfoxide (1.8; 178)
Carbaryl	Ab-bounded magnetic particle	pAb	0.25–5	0.25	2.57	ncr
Carbofuran	Ab-bounded magnetic particle	pAb	0.06–5	0.056	0.815	Metabolites (> 1; > 17)
Chlorpyrifos	Ab-bounded magnetic particle	mAb	0.1–3	0.1	0.94	Diazinon (0.12; 7.56); chlorpyrifos-methyl (0.14; 3.58)
Methomyl	Ab-bounded magnetic particle		0.45–15	0.45	4.15	Thiodicarb (0.49; 11.1)
Benomyl/Carbendazim	Ab-bounded magnetic particle	pAb	0.1–5	0.1	1.6	Benomyl (0.38; 5.61)
Trichlorpyr	Ab-bounded magnetic particle	pAb	0.1–3	0.03	0.78	2-Methoxy-3,5,6-trichlorpyridine (0.01; 1.5)
Trichlorpyrindinol	Ab-bounded magnetic particle	pAb	0.25–6	0.25	2.31	ncr
Chlorothalonil	Ab-bounded magnetic particle	pAb	0.07–5	0.07	1.12	Pentachloronitrobenzene (0.14; 1.9); hexachlorobenzene (0.16; 2.0)
Procymidone	Ab-bounded magnetic particle	pAb	0.8–100	0.8	19	ncr
Captan	Ab-bounded magnetic particle	pAb	10–3000	10	420	Captafol (1000)

^aCross reactivity values are given for the compounds showing the lowest LDD and IC_{50} values. Cross-reactants are listed in order of increasing cross-reactivities. Not all the data reported by the supplier are reported here. ncr means that identified cross-reactivities are out of the linear range.

TABLE 6.2

LIST OF SOME PESTICIDE IMMUNOASSAYS DESCRIBED IN THE LITERATURE (FROM REFS. [13,16,17])

Pesticide	Class	Format	Ab	References
Alachlor	Chloroacetanilide	ELISA	pAb	[24]
Aldicarb	Carbamate	ELISA	pAb	[63]
Aldrin	Organochlorine	RIA	pAb	[64]
Ametryn	Triazine	EIA	pAb	[65]
Aminotriazole	Triazole	ELISA	pAb	[31]
Atrazine	Triazine	ELISAs	pAb, mAb	[13,28,66–78]
		FIA	pAb	[34,78–79]
Benomyl	Benzimidazole	RIA	pAb	[84]
		ELISA	pAb	[85,86,135]
Bentazone	Diazinone	ELISA	pAb	[87]
Bromacil	Pyrimidine	ELISA	pAb	[23]
Captan	Organochlorine	ELISA	pAb	[88]
Carbaryl	Carbamate	ELISA	mAb	[89,90]
Chlorpyrifos ethyl	Organophosphorous	ELISA	mAb	[91]
Chlorothalonil	Chlorobenzonitrile	ELISA	pAb	[134]
Carbofuran	Carbamate	ELISA	pAb	[133]
Chlorsulfuron	Sulfonylurea	ELISA	pAb	[92]
Cyanazine	Triazine	ELISA	pAb	[93]
2,4-D	Phenoxy acid	RIA	pAb	[94,95]
		ELISA	pAb, mAb	[95–97]
		FIA	pAb	[79]
2,4-DB	Phenoxy acid	ELISA	pAb	[97]
De-ethylatrazine	Triazine	ELISA	pAb	[58,72]
Diclofop methyl	Phenoxy acid	EIA	pAb	[98]
Dieldrin	Organochlorine	RIA	pAb	[64]
Difflubenzuron		ELISA	pAb	[99]
Endosulfan	Organochlorine	ELISA	pAb	[100]
Fenitrothion	Organophosphorous	ELISA	pAb, mAb	[101,102]
Fenpropimorph	Morpholine	ELISA	pAb	[103]
Hydroxyatrazine	Triazine	ELISA	mAb, pAb	[70,80–83]
Hydroxysimazine	Triazine	ELISA	pAb	[83]
Iprodione	Chloroanilide	ELISA	pAb	[110]
MCPB	Phenoxy acid	ELISA	pAb	[97]
Metalaxyl	Chloroacetanilide	ELISA	pAb	[104]
Metazachlor	Chloroacetanilide	ELISA	pAb	[105]
Metabenzthiazuron	Phenylurea	ELISA	pAb	[106]
Metolachlor	Chloroacetanilide	ELISA	pAb	[60]
Molinate	Carbamate	ELISA	pAb	[107–109]
Paraoxon	Organophosphorous	ELISA	pAb, mAb	[111,112]
Paraquat	Dipyridinium	RIA	pAb, mAb	[124,125]
		ELISA	pAb	[126]
Parathion	Organophosphorous	RIA	pAb	[113,114]

TABLE 6.2 (CONTINUED)

Pesticide	Class	Format	Ab	References
		ELISA	pAb	[114,115]
Pentachlorophenol	Chlorophenol	ELISA	pAb, mAb	[61,116,117]
Permethrin	Pyrethroid	ELISA	mAb	[118]
Picloram	Carboxylic acid	EIA	mAb	[131,132]
Prometryn	Triazine	ELISA	mAb	[73,120]
Pirimiphos-methyl	Organophosphorous	ELISA	pAb	[102]
Pyrethroids		ELISA	mAb	[119]
Simazine	Triazine	ELISA	mAb, pAb	[83,121,122]
Terbutryn	Triazine	ELISA	mAb	[78]
Terbutylazine	Triazine	ELISA	mAb	[123]
Thiabendazole	Benzimidazole	ELISA	mAb	[127]
Triadimefon	Triazole	ELISA	pAb	[128]
Triazines	Triazine	ELISA	pAb, mA	[71–73]
Triazines	Triazine	FIA	mAb	[77]
Triazoles	Triazoles	ELISA	pAb	[129]
Trifluralin	Nitroaniline	ELISA	pAb	[130]
2,4,5-T	Phenoxy acid	RIA	pAb	[94]

tolerances are often low, typically less than 5–10% for methanol and acetonitrile. They are now given in assay protocols by some companies. It is especially interesting to be able to perform the ELISA in the presence of organic solvent for the determination of pesticides in soil, because the extraction is often carried out with the use of methanol–water. Efforts are made to obtain antibodies which are more resistant to organic solvents. For instance, the assay responses were measured with a recent ELISA developed for the determination of carbofuran in soil and water, and it was shown that recoveries were in the range 102–113% for a methanol concentration in the range 0–10% (v/v), 94% for 20% and 87% for 50% methanol [133].

6.2.4.4. Precision and reproducibility

The precision of an IA is defined as the extent to which replicate analyses of a sample agree with each other. The reproducibility is the ability to yield the same result within analyses, between analyses, and between operators.

According to the non-linear shape of the dose-response curve, the variance is non-uniform, and the experimental errors increase towards the two limits of the measuring range, especially in the non-linear parts. Therefore, the precision should be given by calculating the standard deviation per percent of the coefficient of variation versus concentration. The highest precision is obtained for concentrations close to the concentration obtained at $B/B_0 = 50\%$, representing the concentration of the analyte that gives half of the colour of the negative control, or 50% inhibition, and named IC_{50} . This concentration is often given as a characteristic of tests (see Table 6.1) so that one can estimate the best range for quantitative measurements. However, one

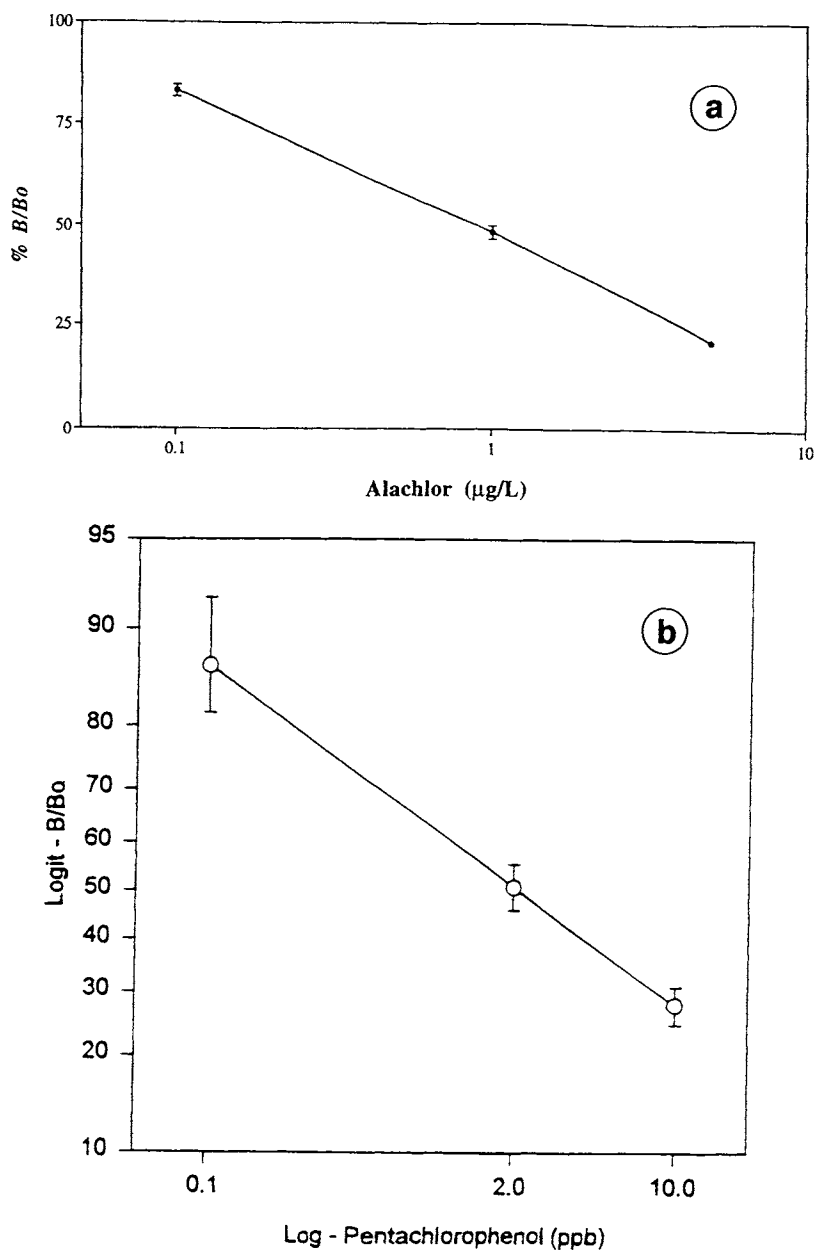


Fig. 6.9. Calibration curves in the working rang. (a) Drawn with % B/B_0 scale, for alachlor. From Ref. [62]. Each point represents the mean of 12 determinations; vertical bars indicate the standard deviation. (b) Drawn with a logit B/B_0 scale, obtained for pentachlorophenol. From Ref. [61]. Each point represents the mean of 79 runs with errors indicating $\pm 2\text{SD}$ from the mean.

TABLE 6.3
PRECISION OF CHLOROTHALONIL MEASUREMENTS BY IMMUNOASSAYS (FROM REF.
[134], WITH PERMISSION)

	Sample ^a			
	1	2	3	4
Replicates	5	5	5	5
Days	5	5	5	5
<i>N</i>	25	25	25	25
Mean ($\mu\text{g/l}$)	0.19	0.50	1.66	3.11
% CV (within assay)	10.6	3.9	4.9	4.1
% CV (between assay)	5.7	6.4	4.6	8.2
% CV (total assays)	11.8	7.1	6.5	8.6

^aWater samples were fortified with 0.25, 0.50, 1.5, and 3.0 ppb chlorothalonil and assayed in five singlets each over 5 days. Sample 1, surface water fortified with 0.25 ppb; sample 2, surface water fortified with 0.50 ppb; sample 3, surface water fortified with 1.5 ppb; sample 4, municipal water fortified with 3.0 ppb.

can see in Fig. 6.9 that the reproducibility of the standard curve in the whole linear part of the measuring range can be good, since the error bars for the two assays represented less than 10% of the coefficient of variation. Most of the studies reported in Table 6.2 state the reproducibility of their assays within and between assays. Table 6.3 shows the precision of chlorothalonil measurements obtained with three types of surface water and one municipal water fortified with chlorothalonil at 0.25, 0.5, 1.5 and 3.0 $\mu\text{g/l}$ [134]. The coefficients of variation within and between assays were less than 11% and 9%, respectively, at the concentration tested. The standard calibration curve for chlorothalonil was linear in the range 0.1–5.0 $\mu\text{g/l}$, with a LDD of 0.07 $\mu\text{g/l}$, as measured at 90% B/B_0 .

6.2.4.5. Specificity and cross-reactivity

The specificity of an assay reflects its ability to produce a measurement of the analyte to be determined. Reliable quantitative determinations using the calibration curve can be obtained provided there is no cross-reactivity. However, since cross-reactivity often occurs in pesticide IAs because of the difficulties of making antibodies for small molecules, it is important to know its effect and to have methods for measuring cross-reactivity.

6.2.4.5.1. Experimental measurements. An experimental measurement of the extent of cross-reactivity is performed by spiking water with related compounds, recording the corresponding absorbance, and drawing the corresponding dose-response curves, similar to the standard curves. Figure 6.10. represents normalized curves measured for atrazine and a few other triazines, using an atrazine ELISA kit. The cross-

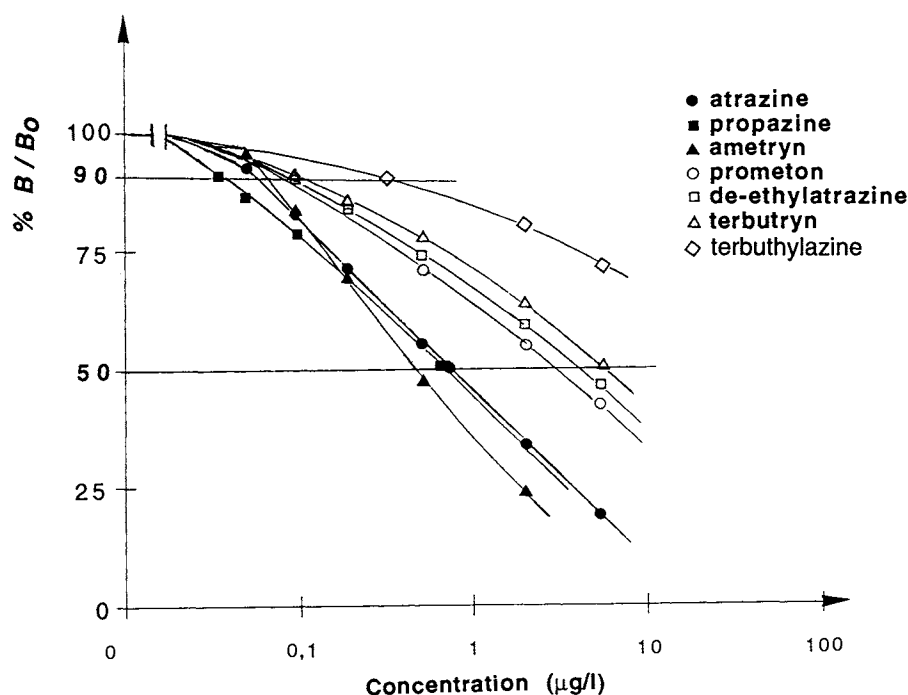


Fig. 6.10. Normalized dose-response curve for atrazine and various cross-reactants, using an atrazine ELISA kit.

reactivity is usually defined as the percent of cross-reactivity according to the following formula:

$$\% \text{ cross reactivity} = \frac{\text{concentration of antigen at } 50\% B/B_0}{\text{concentration of the cross-reactant at } 50\% B/B_0}$$

Instead of giving the % cross-reactivity at 50% B/B_0 , which is also named CR_{50} , one can find the IC_{50} values for both the target analyte and the cross-reacting components. Then, the CR_{50} is the ratio of these two values. However, the CR_{50} values (or the IC_{50} at 50% B/B_0) are not always sufficient to estimate the cross-reactivity because, as shown in Fig. 6.10, the displacement curves of cross-reactants are not parallel to the standard curve over the whole working range. Keuchel and Niessner have recommended the measurement of cross-reactivities at different concentrations over the range where the assay is suitable, and claim that this provide a more realistic estimate of the specificity of the antibodies [136]. This is now accepted, since the LDD values at 90% B/B_0 are given, in addition to IC_{50} values, by companies as characteristics of ELISAs (and are reported in Table 6.1). Table 6.4 gives an example of

the information provided for the characterization of the specificity of an atrazine ELISA. Cross-reactivity is particularly visible for prometon which shows a LDD value of 0.056, close to that of atrazine (0.046), whereas the IC_{50} are respectively 2.22 and 0.72, so that cross-reactivity is higher near the LDD value (CR_{90} 82%) than it is at 50% B/B_0 (CR_{50} 32%). Although ametryn and prometryn show higher cross-reactivities in the middle of the assay than at the LDD values, cross-reactivities are usually higher at low levels than in the middle. The set of the two values, LDD and IC_{50} (or % cross-reactivity), is then useful for a better estimation of the cross-reactivity over the measuring range. Any practitioner can then rapidly estimate the cross-reactivity from data provided with ELISA kits. When one is using IAs for survey purposes over a long period of time, it is useful to know possible cross-reactants, and one just has to check regularly that these compounds are not present in the samples. The kit-selection can also be made according to the target analytes and transformation products. For example, Table 6.1 shows that ELISA kits other than that described in Table 6.4 are available for atrazine. The high-sensitivity magnetic-particle-based IA shows cross-reactivity with propazine and with de-ethylatrazine (CR_{90} and CR_{50} , respectively, of 56% and 25%), whereas the microtitre plates have a low cross-reactivity for de-ethylatrazine (CR_{90} and CR_{50} , respectively, of 20% and 4%). Depending on whether or not the de-ethylatrazine should be included in the survey, one can thus better select the ELISA kit.

Data from atrazine kits are always given as examples of cross-reactivity, but one has to be convinced that most of the ELISA kits designed for compounds other than atrazine are more specific. In Table 6.1, we gave a list of possible cross-reacting

TABLE 6.4

SPECIFICITY OF AN ATRAZINE ELISA FOR VARIOUS TRIAZINE ANALOGUES (ATRAZINE RAPID ASSAY) EXPRESSED RESPECTIVELY AS THE LEAST DETECTABLE DOSE (LDD) WHICH IS ESTIMATED AT 90 % B/B_0 , OR AS THE DOSE REQUIRED FOR 50% ABSORBANCE INHIBITION (50% B/B_0); CR_{90} AND CR_{50} HAVE BEEN OBTAINED AS THE SIMPLE RATIOS OF VALUES FOR CROSS-REACTING COMPOUNDS AND OF ATRAZINE VALUES

Compounds	LDD ($\mu\text{g/l}$)	IC_{50} ($\mu\text{g/l}$)	CR_{90} (%)	CR_{50} (%)
Atrazine	0.046	0.72	100	100
Propazine	0.033	0.74	139	97
Ametryn	0.053	0.39	87	184
Prometryn	0.054	0.64	85	112
Prometon	0.056	2.22	82	32
De-ethylatrazine	0.062	3.21	74	22
Terbutryn	0.090	5.50	54	13
Terbutylazine	0.310	15.5	15	5
Simazine	0.340	4.90	13	15
De-isopropylatrazine	0.800	217	6	0.3
Cyanazine	1.0	>10000	5	—
2-Hydroxyatrazine	1.1	148	4	0.5

compounds, classified in order of increasing cross-reactivity; we have not reported all the data. Generally, the cross-reactivities of many other compounds with closely related structure are reported in the assay characteristics provided by companies. From the data of Table 6.1, it can be observed that many immunoassays show few or no cross-reactivity effects, and can be used for single component analysis. ELISAs for the analysis of pesticides such as cyanazine, chlorsulfuron, paraquat, carbaryl, carbofuran, chlorpyrifos, or methomyl are very specific. Class-selective IAs are available mainly for triazine and phenylurea herbicides.

Regarding cross-reactivity measurements, it is worth mentioning that in real environmental situations, a single analyte is not to be expected, as indicated by commercial ELISAs, but that various analytes will be found together with the target compound. Another important factor in environmental work is the matrix type, which may affect the immunoassays. In this sense, the measurement of the cross-reactivity should approach the real environmental situation as closely as possible. In clinical chemistry, Miller and Valdes [137] reported a model that closely approaches real situations by measuring the cross-reactivity of an analyte in the presence of cross-reactants. It was proposed that one should apply various doses of cross-reactant, each in the presence of various doses of the standard. This approach has been adapted to environmental cross-reactivity studies of immunoassays for chlorpyrifos-ethyl by Oubina et al. [138]. The specificity of the ELISA for chlorpyrifos-ethyl was determined at a fixed standard dose concentration of $0.5 \mu\text{g/l}$ in the presence of various concentrations of cross-reactants and in various environmental matrices. In each experiment, a blank was measured in the absence of the interfering cross-reactant and with only chlorpyrifos at $0.5 \mu\text{g/l}$. The LDD and IC_{50} values were then determined using criteria similar to those reported for standard curves with a single compound. With this variation, a more realistic approximation of the environmental data is performed. This is an advantage over traditional methods where only the cross-reactivity of individual cross-reactants using one matrix is presented, generally an appropriate IA buffer such as phosphate buffer saline (PBS) or ground water. Although the matrix effect is discussed later, the same research group had previously found a great difference between distilled or ground water, compared to estuarine water samples, in the atrazine assay mixed with common cross-reactants [62]. Table 6.5 summarizes the specificity data with the major chlorpyrifos-ethyl cross-reactants. It was found that the response of chlorpyrifos-ethyl was greatest with the lowest IC_{50} concentration of 0.97, 0.98 and $0.99 \mu\text{g/l}$ with distilled, ground, and estuarine water, respectively. Obviously this ELISA kit is not sensitive to the matrix effect with estuarine water. Significant cross-reactivity was thus measured with chlorpyrifos-methyl and pyridifenthion in the three water types. Diazinon, fenotrothion and azinphos-ethyl give much lower cross-reactivity while dichlorvos, temephos and fenthion presented CR_{50} values below 0.01.

Lawruk et al. [139] have described the specificity of the chlorpyrifos RaPid-assay with various organophosphorous pesticides, calculating the % CR_{50} , but each

TABLE 6.5
SPECIFICITY OF THE CHLORPYRIFOS-ETHYL RAPID ELISA IN VARIOUS AQUEOUS MATRICES (FROM REF. [138])

Compounds	Distilled water			Ground water			Estuarine water		
	LDD ^a	IC ₅₀ ^b	CR (%) ^c	LDD	IC ₅₀	CR (%)	LDD	IC ₅₀	CR (%)
Chlorpyrifos-ethyl	0.104 (7) ^d	0.97 (6)	100	0.135 (3)	0.98 (4)	99	0.166 (4)	0.99 (3)	97
Chlopyrifos-methyl	0.33 (6)	7.33 (7)	13.7	0.35 (5)	7.57 (7)	13	0.44 (3)	8.00 (2)	12
Pyridafenthion	0.81 (5)	9.34 (8)	10.6	0.42 (7)	8.11 (8)	12	1.06 (5)	11.86 (7)	8.4
Diazinon	2.82 (5)	32.05 (8)	3.1	4.11 (2)	31.16 (5)	3.2	1.48 (6)	28.69 (6)	3.4
Fenitrothion	463 (6)	2524 (4)	0.04	462 (6)	2318 (6)	0.04	464 (8)	2732 (7)	0.04
Azinphos-ethyl	312 (6)	3297 (7)	0.03	381 (8)	2339 (9)	0.04	185 (3)	2463 (2)	0.04
Dichlorvos	>10000	>10000	<0.01	>10000	>10000	<0.01	>10000	>10000	<0.01
Temephos	>10000	>10000	<0.01	>10000	>10000	<0.01	>10000	>10000	<0.01
Fenthion	>10000	>10000	<0.01	>10000	>10000	<0.01	>10000	>10000	<0.01

^aLDD, least detectable dose calculated at 90% B/B_0 (in $\mu\text{g/l}$).

^bIC₅₀, 50% inhibition concentration (50% B/B_0) (in $\mu\text{g/l}$).

^cCR (%), percentage cross-reactivity is determined by estimating the amount of compound required to displace 50% of the enzyme conjugate to the amount of chlorpyrifos-ethyl.

^dCoefficient of variation expressed as a percentage ($n = 6$ repetitive assays).

TABLE 6.6

COMPARISON BETWEEN THE CHLORPYRIPHOS-ETHYL RAPID ASSAY TEST AND TWO DIFFERENT APPROACHES FOR DETERMINING THE PERCENTAGE OF CROSS-REACTIVITY (% CR₅₀) (FROM REF. [138])

Compound	% CR [139]	% CR [138]
Chlorpyrifos-ethyl	100	100
Chlorpyrifos-methyl	26.2	13.6
Diazinon	12.4	3.11
Fenitrothion	0.02	0.04

time only one compound was measured. Table 6.6 compares the data obtained by measuring the CR₅₀, with a fixed concentration of 0.5 µg/l of chlorpyrifos, with those of Lawruk et al. It should be noted that lower values were obtained with the above method. For chlorpyrifos-methyl, a 13.6% CR₅₀ value was measured using distilled water as compared to 26.2% given by Lawruk et al. [139], and a similar discrepancy was observed for diazinon. For fenitrothion, the CR₅₀ values are not relevant because the curves cannot be precisely determined. This model for checking the cross-reactivity, although more realistic for the analyte's behaviour in the environment, diminishes the CR₅₀ values because of the high specificity of chlorpyrifos-ethyl. Using the same approach with an atrazine ELISA kit, Gascon et al. [62] found CR₅₀ values similar to those reported by Rubio et al. [76] who used the same conventional model as Lawruk et al. [139]. This different behaviour can be explained by a competition process; if the antibody has a high specificity versus the antigen, as in the work of Lawruk et al., then the recognition of the antibody for the different analytes is diminished because the antibody will better recognize its specific antigen instead of the other ones. However, the broad antibody specificity used by both Gascon et al. and by Rubio et al. allows the detection of the majority of triazines, and the CR₅₀ values remain unchanged.

6.2.4.5.2. Relationship with cross-reactivity and antibody characteristics. ELISAs are also used for checking the cross-reactivity of antibodies, and for obtaining a better knowledge of the antibody–interaction reaction. The numerous studies of immunoassays made specifically for atrazine or its metabolites have demonstrated the relationship between the antibodies and the cross-reactivity of the immunoassay.

Thurman et al. [140]] have studied extensively the cross-reactivity from the triazine group using a Res-I-Mun ELISA kit for atrazine. The polyclonal antibodies have been prepared using the hapten shown in Fig. 6.11a [66]. The cross-reactivities are indicated in Fig. 6.12 together with the structures of the cross-reactants [140]. The IC₅₀ values are indicated for each compound. Owing to the hapten design, the chloro-group will not be a determinant group in the recognition and this explains the high cross-reactivity of ametryn. The replacement of an ethyl group by isopropyl in

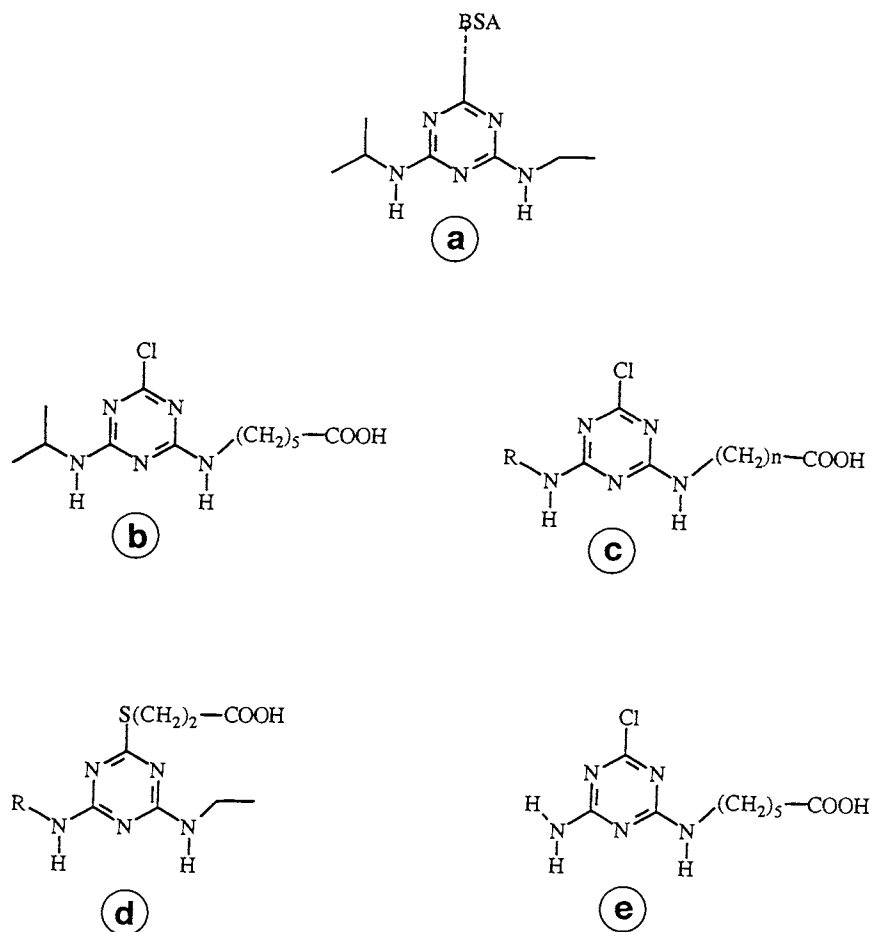


Fig. 6.11. Scheme of haptens used in various ELISAs for the determination of atrazine.

position 2 leads to a slight reduction in recognition, as shown by the cross-reactivity with propazine, prometryn and prometon, whereas the replacement of an isopropyl group by an ethyl in position 1 leads to a low affinity (simazine). Low cross-reactivity is observed with DEA or DIA, certainly because of the different electronic distribution in these molecules caused by the amino group. One might expect this hapten to cross-react with the hydroxy metabolite, but the lower binding can be explained by the difference in structure since hydroxyatrazine also occurs in its keto form. The conclusion is that this kit is class-selective since many triazines can be analyzed together with atrazine, but this does not include the dealkylated metabolites.

Dunbar et al. have used the hapten in Fig. 6.11b to raise polyclonal antibodies [67] The recognition occurs through the chloro and isopropyl groups, thus explaining

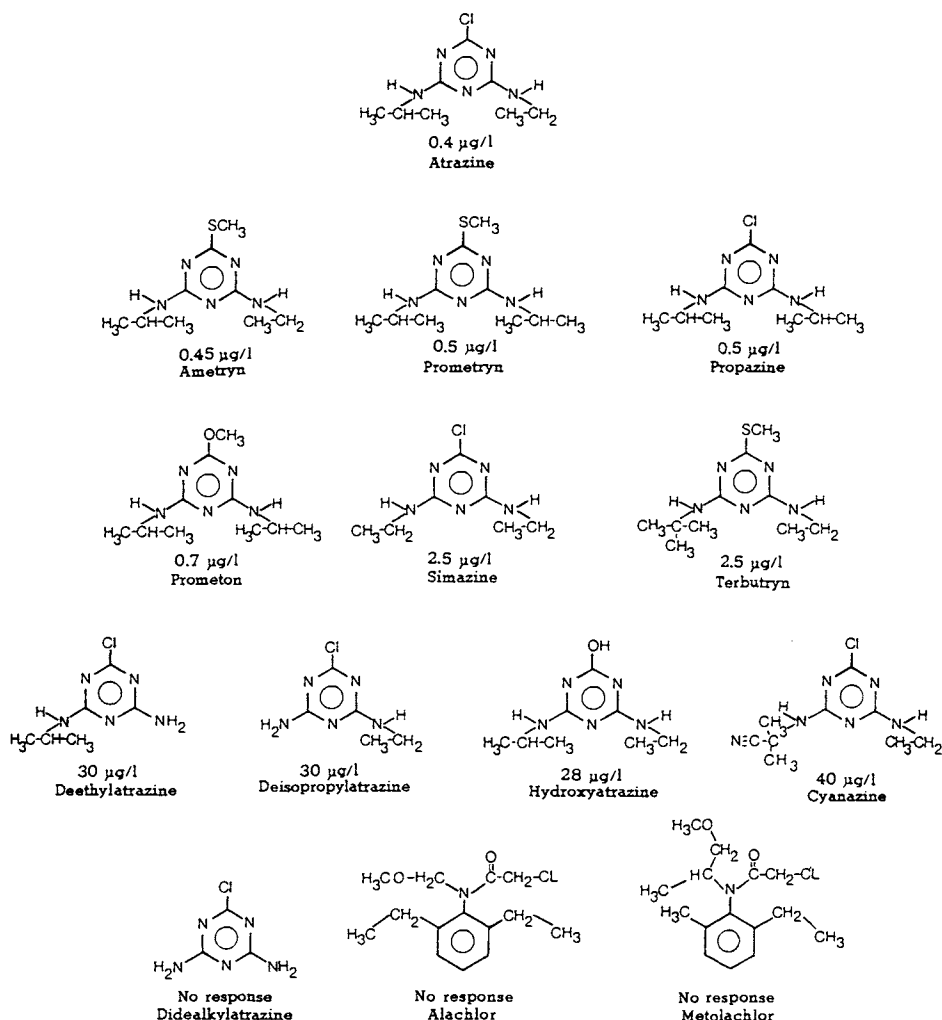


Fig. 6.12. Cross-reactivities observed in a commercial ELISA kit using polyclonal antibodies prepared according to Fig. 6.11a. From Ref. [140], with permission.

the high cross-reactivity measured for propazine (CR_{50} of 87%) and the low cross-reactivity for simazine (10%) and ametryn (7%). The 7% cross-reactivity observed for DEA is certainly explained by the different electronic distribution in the molecule resulting from the replacement of an ethylamino- by an amino group.

Two groups of haptens have been studied by Goodrows et al. (Fig. 6.11c,d) with results in agreement with the previous ones, i.e., a high cross-reactivity with propazine and, to a lesser extent, with simazine for the haptens in Fig. 6.11c but strong cross-reactivity with prometryn, ametryn and symetryn for those in Fig. 6.11d. This

extensive study also pointed out the importance of the length of the spacer [21]. It is clear that the change in electronic density is important for recognition and that the cross-reactivity with metabolites is low, whichever hapten is used for making antibodies against atrazine. Wittman and Hock [72] made ELISA kits for DEA and DIA by selecting a hapten represented in Fig. 6.11e. The response was different for DEA (100% CR₅₀) and DIA (70% CR₅₀), showing a better affinity for DEA having an isopropyl group instead of an ethyl group. This indicated that recognition also occurs for the part of the molecule which is linked to the protein. Muldoon et al. [77] have developed an immunoassay for the analysis of the metabolite chlorodiamino-*S*-triazine. They studied the behaviour of a number of antibodies and confirmed that the antibody recognition of substituted *S*-triazines reduced as a function of amino-side-chain substitution. They also synthesized a diamino-*S*-triazine hapten in order to obtain an appropriate polyclonal antibody for their assay.

Monoclonal antibodies have also been prepared by Schlaeppi et al. [70], using the hapten designed in Fig. 6.11b with a spacer containing four carbon atoms. Although monoclonal antibodies are said to be highly specific, the cross-reactivity with propazine could not be avoided and was as high as that observed with polyclonal antibodies made from a similar hapten. The cross-reactivity with simazine was 2.5% instead of 10% [70]. The same workers also developed monoclonal antibodies for hydroxyatrazine (OHA) with a hapten derived for OHA modified via the ethylamino group. The cross-reactivity was equivalent for hydroxyatrazine and hydroxysimazine. Wittmann and Hock have developed a highly sensitive and specific immunoassay for the analysis of OHA with polyclonal antibodies raised against OHA modified via the ethylamino group [80]. They obtained a LDD of 0.01 µg/l in drinking water, 5–10 times lower than that obtained with monoclonal antibodies raised against the same hapten, and with a much lower incubation time. No cross-reactivity was found with other *S*-triazines, hydroxypropazine or hydroxysimazine a concentration of 5% (v/v) methanol did not affect the assay.

Lucas et al. [82] compared the properties of ELISAs kits made with two monoclonal and polyclonal antibodies raised against the same hapten, shown in Fig. 6.11d, by modifying atrazine via its chloro group and with polyclonal antibodies raised against the hapten in Fig. 6.11a via atrazine's ethylamino group. The most sensitive assays have been obtained with one of the monoclonal antibodies, and these were shown to be more resistant to organic solvents. The cross-reactivity was only measured for simazine and was 61% and 67% for the two monoclonal antibodies, whereas it was found to be 12% for the polyclonal antibodies derived from the same hapten and 20% for the second polyclonal antibodies.

All the studies reported above indicated that the specificity of the IAs is not always much higher when using monoclonal instead of polyclonal antibodies. Especially when polyclonal antibodies show high cross-reactivity, it is likely that the monoclonal ones will also show some cross-reactivity. The difficulty of making a very specific atrazine antibody results mainly from the closely related structures of

several compounds within the triazine group, because many other chlorotriazines have two common determinants with atrazine. Highly specific ELISAs have been made with monoclonal antibodies for terbutylazine [123] or with polyclonal antibodies for cyanazine [93], but both terbutylazine and cyanazine have determinants which are less common with other triazines. Although it is often claimed that monoclonal technology provides antibodies with low cross-reactivity, many IAs are very specific when polyclonal antibodies are used. However, antibodies with high cross-reactivity have helped in the development of ELISAs for screening the group of triazines. Antibodies with high cross-reactivity have also been obtained for some phenylurea herbicides, so screening ELISAs are also available for this group (see Table 6.1).

6.2.4.6. Accuracy

The accuracy of an assay reflects its ability to measure true values for an analyte. The dose-response curves are usually constructed with standard solutions provided with the kit, which are in buffered solution with preservative and stabilizers. A zero control solution is also provided, to be used as diluent if necessary. A control solution is also provided in kits, which is to be treated in the same manner as an unknown sample, and allows one to test the accuracy of the ELISA. Another easy test consists of preparing several dilutions of a real sample and simply measuring them. Ideally, in the absence of interfering substances, the standard curve should be parallel to the curve obtained by diluting the sample within the working range. Another test is to calculate recoveries by spiking real sample with known amounts of the target analytes. However, the best evaluation of the accuracy is obtained by comparing the results obtained from real samples using other validated analytical techniques.

6.2.4.7. Matrix effect

The range of detection levels of pesticides found in real water samples implies their analysis without any sample pretreatment (except dilution in buffer) in most cases. Therefore it is important first to know whether the calibration curves constructed with standard solutions can be used with real samples. Because the assay is based on competitive interactions between antibodies, analytes, and labelled analytes, it can be affected by the pH or the ionic strength of real water matrices. Interferences originating from the presence of structurally related compounds in the samples can lead to false positive responses of the IAs. It has also been shown that the presence of other synthetic or natural substances, including halogens or dissolved organic carbon, that interact weakly with the antibody but are present at sufficiently high concentrations, can also give positive values in the assay without the presence of the analyte [141,142]. When various types of water are analyzed, if the matrix variation has a strong effect on an immunoassay, then the assay is more appropriate for screening purposes rather than for accurate quantitation. As with cross-reactivity,

the matrix effects can be different and a substance that interferes with one IA system may interfere to a much different degree in another one.

6.2.4.7.1. Effect of the water type and ionic strength. The direct application of an IA to the matrix of interest can be troublesome. Guidelines included in every kit recommend that samples containing gross particulate matter should be filtered and that samples which have been preserved with acids should be neutralized prior to assay.

The matrix effect can be investigated by constructing dose-response curves with real samples spiked within the working range, and including a blank run, or by measuring the recoveries for real samples, spiked at known levels, using the standard curve constructed with the standard solutions provided within the kit. With the former method a shift to the right or to the left, whilst keeping parallelism, corresponds to an increase or a loss in the sensitivity and may indicate the need of performing measurements by preparing the standard curves with the matrix. Figure 6.13 gives an example, in which waters from different sources were analyzed [143]. No significant differences in slope and IC_{50} were observed when running the carbaryl ELISA in river, tap or well water compared with the standard curve obtained for the experiment carried out in buffer. These data show that no clean-up is necessary and that water samples can be used directly with IAs. In general, the recoveries measured

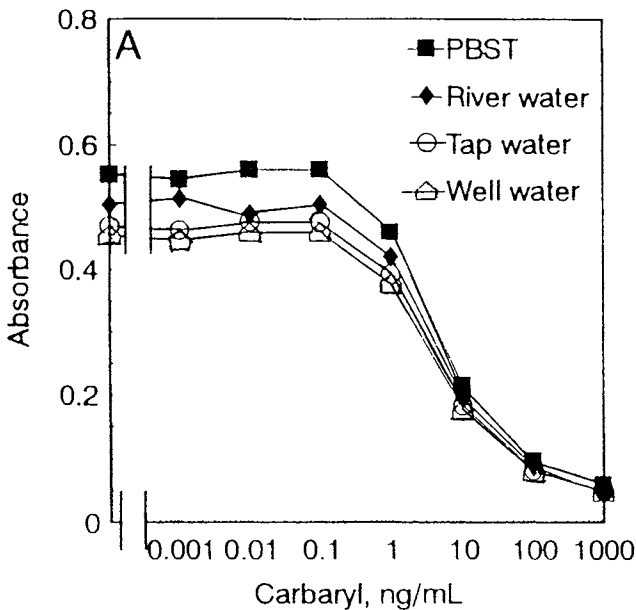


Fig. 6.13. Matrix effect studies with water from several sources, using a carbaryl microtiter plate ELISA. From Ref. [143]. Each point represents the average of duplicate determinations (PBST is a phosphate buffer saline with 0.05% Tween 20).

with spiked surface water samples indicate that no matrix effects are present in ELISAs. As an example, 326 drinking, surface, and ground water samples obtained throughout the USA were fortified with 1.5 $\mu\text{g/l}$ of metalochlor to evaluate the sample matrix effect [60]. The metalochlor concentrations of the water samples ranged from less than 0.04 up to 4.54 $\mu\text{g/l}$ before they were spiked. The experimental recoveries ranged between 76 and 120%, with a mean recovery of 96% (SD = 9%).

No interferences from the matrix have been observed in the analysis of paraquat in agricultural waste water samples [126]. In the same study, an application to biological samples was also described for screening the worker exposure, and plasma or urine could be analyzed directly by 1:1 dilution with PBS with a limit of detection of 2 ng/ml for whole plasma. Lymph was a more complicated matrix especially because of interferences from protein but, with preincubation of the samples with BSA, the determination of paraquat using ELISA showed only slight protein interferences. It is worth noting that ELISAs have found many applications for the determination of environmental pollutants in human biomonitoring, and an excellent review has been published by Knopp [144]. This author noted that the more polar analytes could be measured with reduced or no sample preparation in biological samples, whereas hydrophobic chemicals such as dioxins and PCBs, make extraction and some sample clean-up necessary in order to reduce interferences by matrix constituents.

Whenever a matrix effect influences too much the competition reaction occurring in the ELISA, seen in a non-parallel dose-response curve, one is recommended to develop a simple sample-preparation method. As the sample volume required is very small, LLE or SPE can easily be performed. For compounds with a low solubility in water or with a high lipophilicity, their tendency to stick everywhere, and not especially on the antibody, can often be overcome, by the addition of small amounts of organic solvent or detergents [16]. However, the organic solvent or detergent can modify the standard curve and it may be necessary to construct a dose-response curve with the addition of the same amount of organic solvent to the matrix samples. Two ELISAs were recently described for the determination of PCBs in water and soil. One of these could easily accept up to 20% of methanol [145]. For the other, one is recommended to dilute the water samples with 50% methanol immediately after collection; the standards were also prepared in an acetate-buffered saline-preserved solution containing 50% methanol for constructing the standard curve [146].

The examples mentioned above, and many others reported in the literature dealing with various types of aqueous matrices, indicate that the ionic strength, although different in every sample, does not affect the dose-response curve too much. It is common to find in the guidelines or publications for commercial kits the statement that ELISAs are not affected by the addition of nitrate, thiosulfate, sulfite, sulfide, copper, iron, nickel, calcium, or magnesium, at concentrations up to 250 mg/l. The addition of sodium chloride shows no effect on results up to concentrations of 0.6–1 M, which indicates that sea or estuarine water samples can be analyzed directly, but very

few studies report the determination of pesticides in such waters. Oubina et al. [138, 142] have shown that the matrix of estuarine water did not affect the standard curve in the chlorpyrifos-ethyl assay. Estuarine water have also been analyzed for atrazine and alachlor using ELISAs; no effect was observed for alachlor, but some reduction in response was observed for atrazine [62,147–149]. Table 6.7 gives the LDD, IC₅₀ and CR₅₀ values for atrazine and various cross-reactants in buffered distilled water, ground water and estuarine water, and for alachlor and its possible cross-reactant metalochlor [62]. For the alachlor ELISA, both the LDD and CR₅₀ values are similar in every water, and it is shown that metalochlor does not cross-react with a LDD above 5 µg/l. The situation is different for the response of atrazine. The CR₅₀ values calculated according to values obtained in buffered distilled water are close to 100% for atrazine in ground water but are somewhat lower in estuarine water. For such types of water, and with this atrazine ELISA kit, there is a matrix effect which is also observed for the cross-reactant analytes, probably because of the presence of humic acids in these waters. As a consequence of this matrix effect, false positive results were given in the reported survey of atrazine in estuarine waters when a comparison was made with GC analyses [148].

6.2.4.7.2. Effect of humic acid content. The effect of the humic acid content of samples is often studied by spiking samples with a known amount of commercially available humic acids. The addition of humic acids up to 50 mg/l caused no

TABLE 6.7
SPECIFICITY OF ATRAZINE AND ALACHLOR ELISAS IN DIFFERENT AQUEOUS MATRICES
(FROM REF. [62])

Compounds	Distilled water			Ground water			Delta water		
	LDD ^a	IC ₅₀ ^b	CR (%) ^c	LDD	IC ₅₀	CR (%)	LDD	IC ₅₀	CR (%)
Atrazine	0.05	0.30	100	0.05	0.30	100	0.08	0.40	75
De-ethylatrazine	0.07	0.37	81	0.09	0.46	65	0.10	0.49	62
De-isopropylatrazine	0.41	2.06	15	0.45	2.27	13	0.44	2.19	14
Simazine	0.35	1.75	17	0.40	2.09	14	0.42	2.10	14
Alachlor	0.05	0.29	100	0.05	0.29	100	0.05	0.29	100
Metolachlor	5.34	26.7	1.1	5.34	26.7	1.1	5.4	26.7	1.1

Atrazine and alachlor RaPID ELISA for each aqueous matrix.

^aLDD, least detectable dose calculated at 90% B/B₀ (in µg/l).

^bIC₅₀, 50% inhibition concentration (50% B/B₀) (in µg/l).

^cCR (%), percentage cross-reactivity is determined by estimating the amount of compound required to displace 50% of the enzyme conjugate, divided by the amount of atrazine or alachlor needed for 50% displacement.

interference from the samples for the ELISA determination of carbofuran in water [133], whereas the addition of 5 mg/l of humic acids was reported to generate non-specific interactions with antibodies in an ELISA kit for the analysis of trinitrotoluene [16]. As a consequence, a serious depression of the standard curve was observed. After treating the samples with BSA, no influence of humic acids was observed because the pre-incubation step with BSA preserved the antibodies from such interferences [16]. Large amounts of humic acids can occur when aqueous soil extracts are introduced into some IAs. However, when experiments have shown that the addition of humic acids has no significant effect, this is notified in commercial ELISA kits.

On the other hand, although it is important to study the effect of humic acids for the ELISAs, it is also well known that humic acid determination is a relevant problem for environmental chemists working in soil science or hydrochemistry. Immunoassays have thus been developed for dissolved humic acids [16]. Although the individual peculiarities of the humic acid molecules are not known, a humic acid antibody could be raised, allowing the determination of humic acids in the sub- $\mu\text{g/l}$ range.

6.2.4.7.3. Effect of the sample pH. The pH of natural waters does not usually affect the immunoassays. No difference in the chlorpyrifos determination was observed when samples were at pH 4 or at pH 8 [142]. Figure 6.14a shows the standard curves run at different pH values with a commercial atrazine ELISA kit [149]. In this study, IAs could be performed without sample preparation with Ebro water of pH near 8. A slight bias on the standard curve was found at pH 2. These results are in agreement with those presented in Fig. 6.14b, where it is shown that no change in response is observed at pH 2 but a dramatic change occurs at pH 1. This can be explained by the atrazine's ionization, which occurs below pH 2 (pK_a around 1.6). For the same reason, ELISAs for the determination of 2,4-D were reported not to work with samples acidified to pH 2 [150]. One can expect the same effect for hydroxyatrazine (pK_a around 4.6); no significant differences were observed in its standard curves for pH varying in the range 5.0–8.5 [83].

6.2.4.7.4. Effect of filtration of the samples. Filtration was shown to have no effect on the analysis of 2,4-D in river Rhine water [150], and no significant difference was observed between filtered and unfiltered estuarine waters in the determination of chlorpyrifos-ethyl at the 0.5 $\mu\text{g/l}$ level [142]. This compound has an intermediate behaviour, with a $\log K_{oc}$ of 3.4, lower than typical compounds which may adsorb onto the particulate matter, such as PAHs or PCBs which have $\log K_{oc}$ in the range 5–9. Its behaviour and determination were therefore not affected too much by the presence of particulate matter [151]. However, the effect of particulate matter can be important when the pesticide concentration is increased.

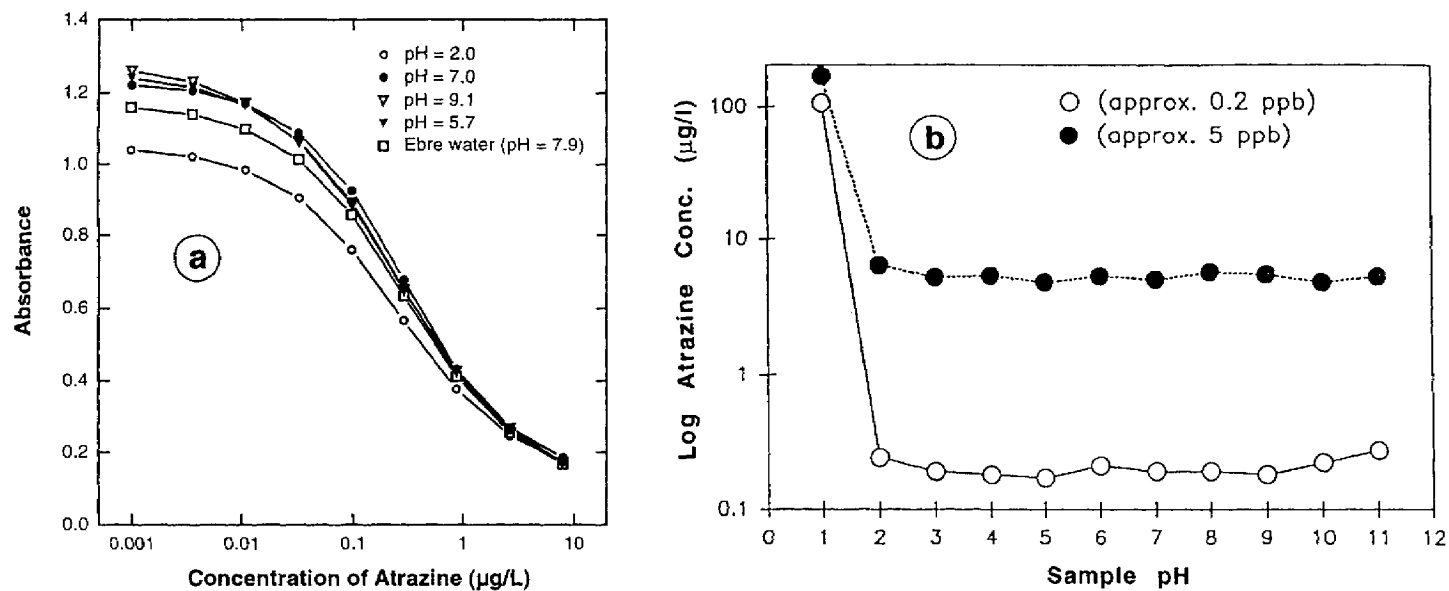


Fig. 6.14. Effect of sample pH on: (a) the dose-response curves run at different pH values, using the particle-based high-sensitivity atrazine immunoassay. From Ref. [149]; (b) the apparent atrazine concentration observed with samples containing approximately 0.2 or 5 $\mu\text{g/l}$, using a particle-based atrazine ELISA. From Ref. [76], with permission.

6.2.5. Data interpretation, effect of cross-reactants in samples, and comparison with chromatographic methods in validation studies

When a value is measured in an unknown and non-spiked sample, using the standard curve, one question to be asked is, “How can we be sure that this value corresponds to the amount of the analyte?” As seen above, any matrix effects resulting from the water type can be checked by spiking this sample with known amounts of analyte. However, also as pointed out above, some IAs are sensitive to cross-reactants which may be present in the unknown sample and have a strong effect on the measured value. Therefore, special care must be taken in data interpretation.

6.2.5.1. Analyte equivalency

One must be aware that IAs do not measure the concentration of an analyte but that of an indicator species giving absorbance in a coloured solution. This results from a competition reaction between the “analyte-antigen” and the labelled analyte. For an unknown sample, the sample response is compared to standard responses obtained with calibrators made of solutions spiked with the analyte antigen. The conclusion from an IA is that, “a certain amount of the “analyte antigen” is present in the sample. In fact, it is more correct to say that the measurement corresponds to an “equivalent of the analyte antigen”.

Figure 6.15 simply illustrates the problem of data interpretation with real samples according to the presence of cross-reactants in samples and their effect on the ELISA kit. It shows first the results given by an atrazine ELISA kit, i.e., the concentration in atrazine equivalents, upon the addition of increasing amounts of de-ethylatrazine (Fig. 6.15a), de-isopropylatrazine (Fig. 6.15b), and simazine (Fig. 6.15c) to real samples each initially containing $0.5 \mu\text{g/l}$ of atrazine. Figure 6.15d shows the results given by an alachlor ELISA kit when increasing amounts of metalochlor were added [62]. First, the matrix effect can be seen, since the concentration of distilled water and ground water samples spiked at the $0.5 \mu\text{g/l}$ level, with the addition of $0.01 \mu\text{g/l}$ of cross-reactants, indicates an “atrazine equivalent” around $0.60 \mu\text{g/l}$ ($\pm 0.03 \mu\text{g/l}$), whereas these values are higher with estuarine water spiked at the same levels of atrazine and cross-reactants. From the various compounds studied, one should note the higher deviation observed for de-ethylatrazine as than for all the other analytes, which thus indicates a higher influence of this commonly detected transformation product in the atrazine determination, using this kit. We can also see that when the concentration of the cross-reacting analytes increases, the slope has a higher value and consequently a higher deviation from the original concentration is obtained. With the alachlor ELISA, the addition of metalochlor up to $1 \mu\text{g/l}$ has no visible effect since the resulting alachlor concentration varies within $0.02 \mu\text{g/l}$. This result confirms the low cross-reactivity (1%) of metalochlor provided by the antibody used in the alachlor ELISA.

For a given amount of the added cross-reactants in the original samples, i.e.,

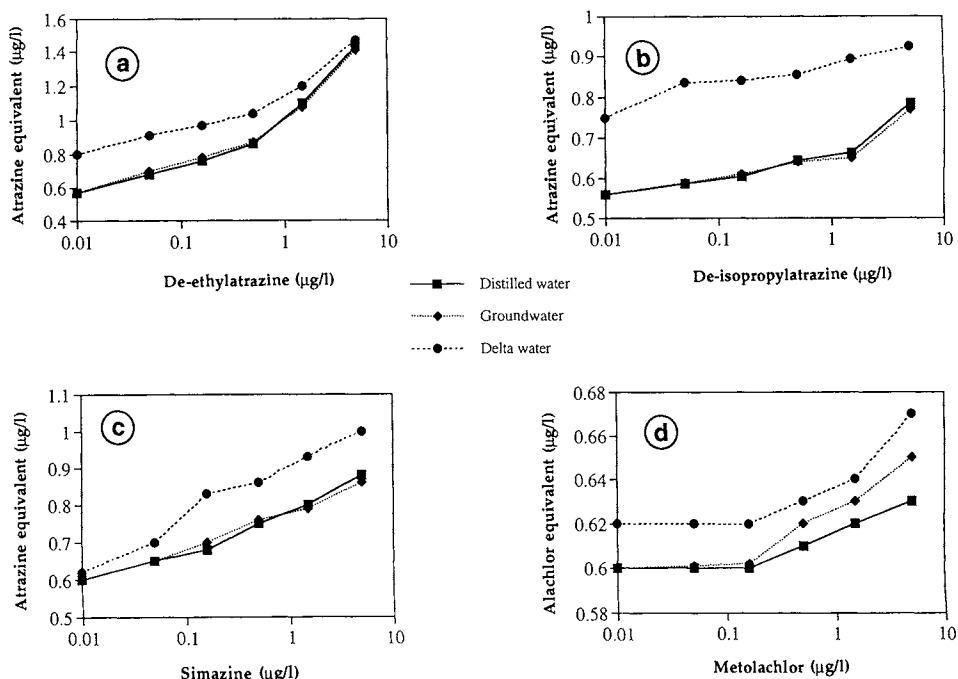


Fig. 6.15. Dose-response curve for atrazine and alachlor at $0.5 \mu\text{g/l}$ in the presence of (a) de-ethylatrazine, (b) de-isopropylatrazine and (c) simazine (for the atrazine ELISA kit) and (d) metolachlor (for the alachlor ELISA kit). The concentrations of the cross-reactants are 0, 0.05, 0.16, 0.5, 1.5 and $5 \mu\text{g/l}$ in buffered distilled water, ground water and estuarine water. From Ref. [62].

$0.5 \mu\text{g/l}$, the “atrazine-equivalents” measured in ground water samples are $0.85 \mu\text{g/l}$, $0.64 \mu\text{g/l}$, and $0.76 \mu\text{g/l}$ when de-ethylatrazine, de-isopropylatrazine and simazine, respectively, are added. These values depend on the cross-reactivity properties of the kit (see the ELISA specificity in Table 6.7). Only cross-reactants with a CR value of 100% should give an atrazine equivalent of $1 \mu\text{g/l}$ corresponding to the sum of the atrazine and cross-reactant concentrations. This explains why one should take care in data interpretation using triazine kits. They can only provide an “atrazine-equivalent” which has to be considered in the light of a close examination of the cross-reactivities of the kit when the samples are unknown. Owing to the variable cross-reactivity of the triazines, when several triazines or metabolites are present in the samples, the measured “atrazine-equivalent” cannot be equal to the true concentration of the total triazines, and is always lower. This is shown in Fig. 6.16a which represents the correlation between the “atrazine-equivalent” given by an atrazine ELISA kit and the total triazine concentration measured by GC-NPD in estuarine water samples, all containing atrazine and simazine, and one also containing de-ethylatrazine [147]. Owing to the cross-reactivity, measured in the

estuarine matrix, of 14.4% (CR₅₀) for simazine and 61.7% for de-ethylatrazine, it is easy to explain why the “atrazine-equivalent” is always lower than the total triazine concentration measured by GC-NPD (slope of 0.58). From the respective concentrations measured by GC-NPD, the authors consider that the “atrazine-equivalent” was obtained by adding the concentration of atrazine in the sample to those of simazine and de-ethylatrazine, modified by the cross-reactivity values. They were then able to calculate the value given by the ELISA which was owing to atrazine. Figure 6.16b shows the good match between this calculated value and the concentration of atrazine measured by GC-NPD. These results provide an understanding of the mathematics that can be applied for data interpretation and show the necessity of knowing the cross-reactivity in order to predict the effect of the concentrations of the various triazines and/or metabolites to the “atrazine-equivalent” number. This can be a help, for example, in estimating the level above which samples will have to be analyzed by GC for a regulatory purpose.

The results presented above indicate that the data interpretation for unknown samples requires a good knowledge of the characteristics of the kits, and explains why data interpretation for real samples is linked to validation studies by chromatographic techniques.

6.2.5.2. *Comparison with chromatographic measurements in validation studies*

The validation of results given by IAs is usually performed by comparison with chromatographic methods, when available, and if possible with real samples. Environmental surveys provide a good opportunity, and the group of M. Thurman (US Geological Survey) was among the first to use immunoassays and gas chromatography-mass spectrometry in a reconnaissance study of herbicides and their metabolites in the midwestern United States [151]. In the section below, we report some selected results of validation studies, classified by their IA characteristics and whether cross-reactants can be found in the sample matrix or not.

6.2.5.2.1. No probability of cross-reactants in the samples. From the characteristics given by the companies with their kits, the user can easily obtain an idea of the cross-reactants that have been identified so far and the probability of their being present in the sample. When possible, validation of IAs is better performed using real samples contaminated by the analyte. This can even give an opportunity for identifying new cross-reactants and metabolites, as was the case for alachlor. The ethanesulfonic acid (ESA) metabolite was identified in real samples as a result of the frequency of false positives which were observed by using the ELISA screening kits for alachlor and have been attributed to the significant cross-reactivity of the ESA metabolite towards the anti-alachlor antibody [152,153]. Aga et al. [154] applied a sample pretreatment for measuring alachlor and the ESA metabolite separately with the alachlor kits and it has recently been shown that the median ESA concentration exceeded the median concentration of alachlor in some rivers and reservoirs [155,156].

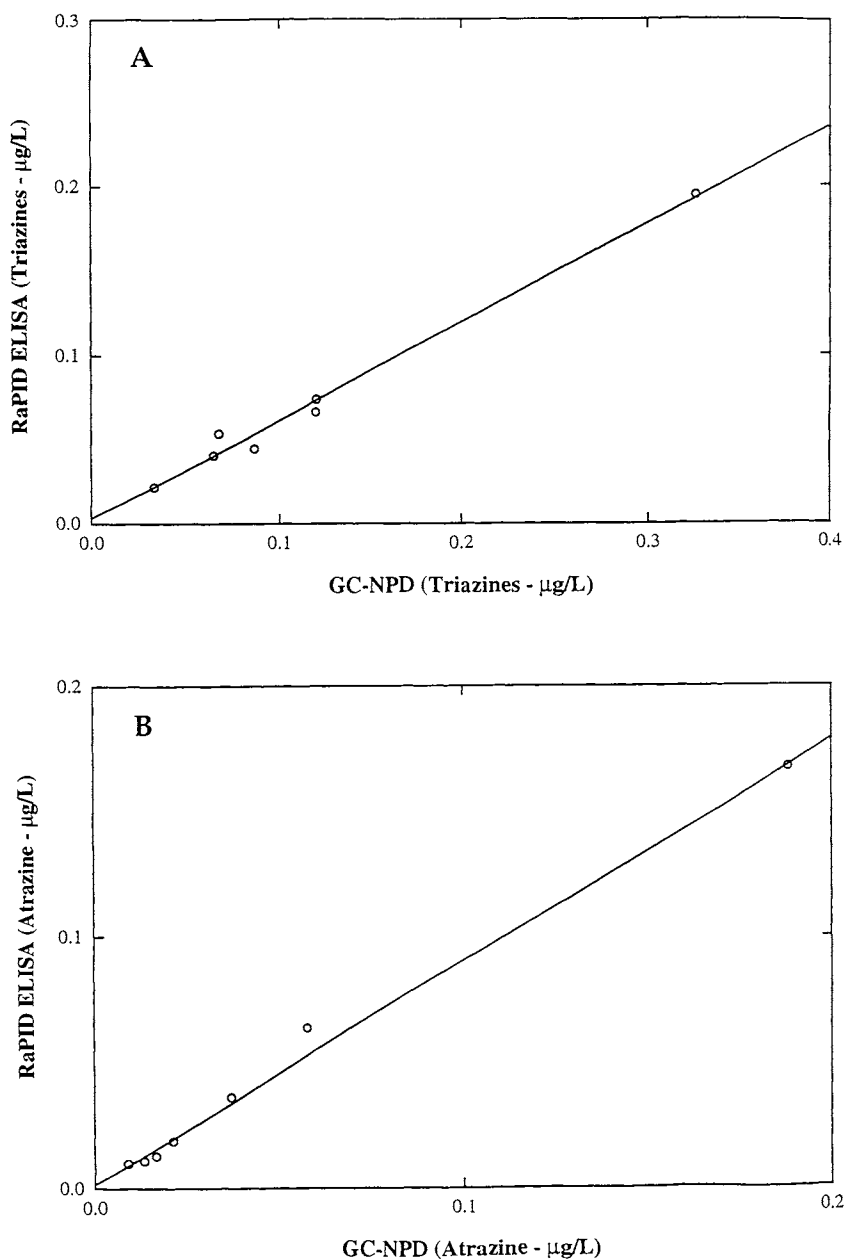


Fig. 6.16. (a) Comparison of the "atrazine-equivalent" given by the RaPID ELISA kit and the total triazines (mainly atrazine and simazine) measured by GC-NPD in estuarine samples from the Ebro delta (Tarragona, Spain). $n = 7$, $r = 0.994$, $y = 0.583x + 0.0031$; (b) comparison with the calculated value of atrazine concentration given by the ELISA and the atrazine concentration measured by GC-NPD. $n = 7$, $r = 0.997$, $y = 0.894x + 0.0024$. From Ref. [147], with permission.

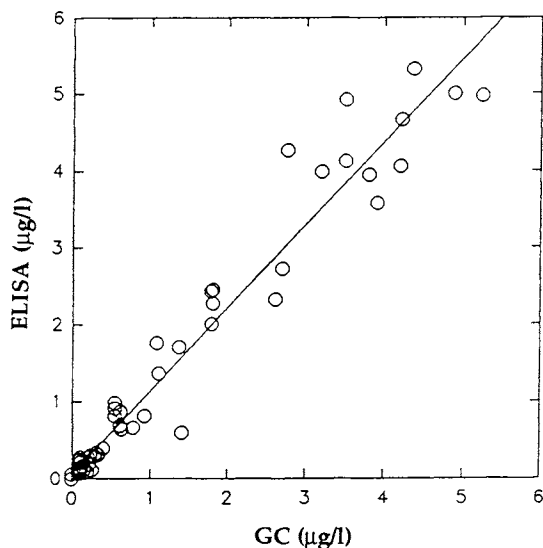


Fig. 6.17. Correlation between metalochlor concentrations as determined by the magnetic particle-based ELISA and GC methods. $n = 58$, $r = 0.976$, $y = 1.08x + 0.063$. The 58 water samples were drinking, surface, and ground waters from various locations in the USA and Canada. From Ref. [60], with permission.

In another study, it was possible to correlate the concentrations of real samples contaminated by metalochlor (one of the most widely used herbicides in the US and Canada), as determined by an ELISA, with those from GC methods, as shown in Fig. 6.17 [60]. The regression analysis between the methods yielded a correlation of 0.976 and a slope of 1.08. According to the authors, the apparent higher metolachlor sample concentrations could result from cross-reactivity of the antibody with metabolites and other chloroacetanilides (but not confirmed by the GC analysis) or could be explained by a loss of analyte during the sample preconcentration step of the GC method.

ELISA kits for the determination of carbofuran show no significant cross-reactivity towards the known metabolites. The correlation was correct ($r = 0.967$, slope of 1.18) between ELISA measurements and LC methods which involved an extraction and concentration step, a post-column derivatization, and fluorescence detection for ten naturally contaminated samples at concentrations within the 1–4 $\mu\text{g/l}$ range [133].

For pesticides which are less commonly detected in environmental water, validation studies are performed with spiked samples. Good correlations have been found using ELISA for the determination of chlorothanil as compared with GC-FID methods [134], and for carbendazim (or benomyl after transformation into carbendazim) as compared with LC determinations [135]. Oubina et al. [142] evaluated an ELISA

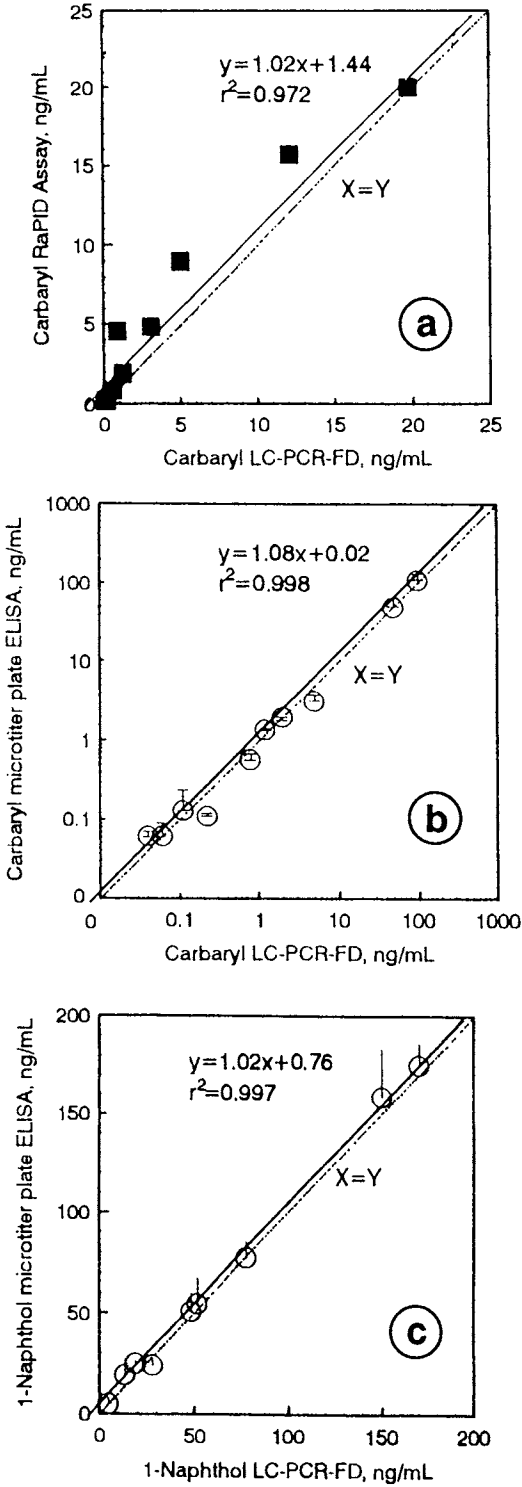
for the determination of chlorpyrifos-ethyl and compared the results given by IA with those obtained by automated on-line solid-phase extraction (Prospekt) followed by LC-DAD in spiked estuarine waters in the range 0–4 $\mu\text{g/l}$. The regression yielded a correlation of 0.991 with a slope of 0.107. Marco et al. [143] validated two immunoassay methods for the environmental monitoring of carbaryl and 1-naphthol in ground water samples. Figure 6.18 shows the correlation between LC determinations with post-column derivatization and fluorescence detection (LC-PCR-FD, according to the EPA method 531.1) and two ELISA formats for the determination of carbaryl and one for the determination of *l*-naphthol. The slope values of the linear regression equation were in all cases near to 1 and the correlation coefficients were always higher than 0.95.

6.2.5.2.2. Probability of cross-reactants in the samples. Most examples of the use of ELISAs, performed with non-spiked samples, and which compare the ELISA results with those obtained by chromatographic techniques, are devoted to atrazine, since this herbicide is found everywhere in the world and is, of course, included in most environmental surveys [14,70,148,149,151,154–160].

Thurman et al. [151], using a microplate atrazine ELISA, whose characteristics (CR_{50}) have been given in Fig. 6.12, plotted the “atrazine-equivalent” versus the concentration of atrazine obtained by GC-MS for 127 surface water samples. The kit cross-reacted with ametryn, prometryn, prometon, propazine, simazine and terbutryn, but not with cyanazine and the degradation products of atrazine at the typical concentrations found in water samples. As the GC-MS indicated that cyanazine and de-ethylatrazine were frequently detected with trace concentrations of simazine and propazine, the agreement between ELISA measurements and GC-MS was good, especially for atrazine concentrations lower than 5 $\mu\text{g/l}$. Using a similar kit, Ferguson et al. [14] has found a good correlation for the atrazine concentration measured by ELISA and by GC-MS in contaminated wells at rather high concentration levels in the range 1–40 $\mu\text{g/l}$. Regression analysis yielded a correlation coefficient of 0.996 and a slope of 1.2 between the methods, indicating a slight ELISA bias.

The particle-based atrazine ELISA has different characteristics and cross-reacts to a greater extent with dealkylated products. The fact that de-ethylatrazine exhibits a high cross-reactivity is useful in atrazine surveys, since the relationship between de-ethylatrazine and atrazine is a measure of the usage of atrazine and gives an indication of the period of application. In certain cases, the value for de-ethylatrazine is even higher than that for atrazine. In contrast, de-isopropylatrazine is always de

Fig. 6.18. Correlation between LC-PCR-FD and ELISA for three assays. Samples were spiked and analyzed simultaneously by both methods. Regression equations and correlation coefficients are shown in the graphs for each experiment: (a) LC-PCR-FD/RaPid assay for carbaryl, (b) LC-PCR-FD/microtitre-plate ELISA for carbaryl, (c) LC-PCR-FD/microtiter-plate ELISA for 1-naphthol. From Ref. [143].



tected in a much smaller amounts than de-ethylatrazine, since its formation by a microbiological process is less favoured than the formation of de-ethylatrazine. In the last few years, much more atrazine has been detected in combination with de-ethylatrazine and simazine in natural waters [151,155,156,161–166]. Therefore, the magnetic particle-based ELISA kit studied in Figs. 6.15 and 6.16 is more appropriate for monitoring the total chlorotriazines, although the line in Fig. 6.16a has shown that the measured concentration of total chlorotriazines is lower than the real one. On the another hand, when the correlation of the “atrazine-equivalent” is made with the GC-atrazine concentration, the ELISA values overestimate the real concentration of atrazine. Rubio et al. have obtained a good correlation ($r = 0.971$, $n = 41$) using the particle-based ELISA and GC-MS determination of atrazine in contaminated ground water samples [76]. The higher slope obtained by the ELISA (1.45) certainly resulted from the occurrence of de-ethylatrazine, which was not monitored in the survey and can be high in ground water [166]. Another example is given in Fig. 6.19 which corresponds to a survey of 750 water samples collected from four Vermont streams in the USA, with a set of 224 of them which have been also analyzed by GC-MS [157,158]. No false negative was observed and only 5.5% of the assays gave a false positive using ELISAs. The authors explained the over-estimation by the presence of cyanazine which was found in 35% of the samples analyzed by GC-MS and by the probable presence of de-ethylatrazine, but no measurement was made. When looking at the low cross-reactivity of the kit with cyanazine, the second reason is certainly most likely.

Another relevant example of the effect of cross-reactants in the sample is in the determination of atrazine in freeze-dried water samples, which have been prepared in collaboration with the Community Bureau of Reference of the Commission of the European Community with the objective of obtaining a candidate reference material containing atrazine, simazine and other pesticides [148]. Such samples were obtained from water samples to which 0.6% of glycine had been added as stabilizer. Two batches were analyzed using the atrazine magnetic-based ELISA kit and by GC-MS, before and after liquid–liquid extraction and Florisil clean-up. Table 6.8 gives the concentration of atrazine measured by the ELISA and the concentration of atrazine and simazine determined by GC-MS. There is an important matrix effect owing certainly to the addition of glycine to the samples, and a clean-up step was introduced to eliminate this matrix effect. Two different results are obtained: in batch A, the ELISA gave a value of $0.78 \pm 0.08 \mu\text{g/l}$ which corresponded to the sum of $0.62 \mu\text{g/l}$ of atrazine in the presence of $1.89 \mu\text{g/l}$ of simazine (three times the concentration of atrazine), a result which is easily explained and is in agreement with Fig. 6.15, owing to the cross-reactivity of simazine. In Batch B, the value given by the ELISA corresponds exactly to the atrazine concentration measured by GC. This is explained by the higher atrazine concentration compared to batch A, and the lower concentration of simazine, so that the effect of the simazine is negligible.

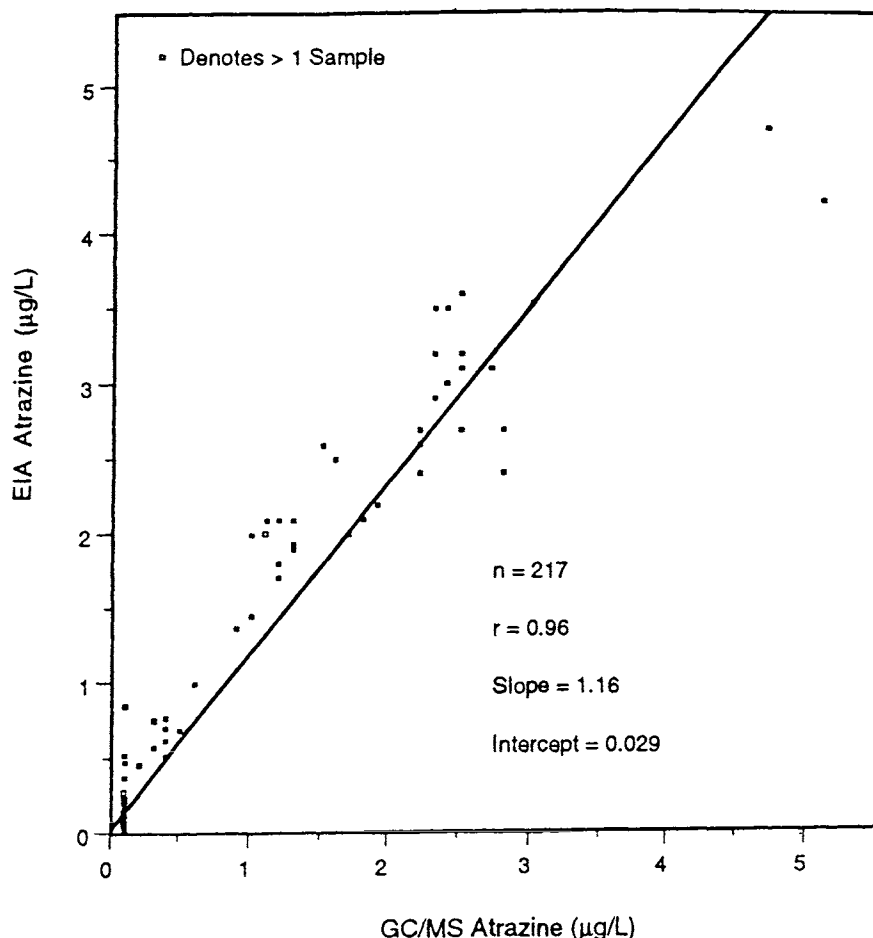


Fig. 6.19. Regression comparison of stream water atrazine concentration as determined by immunoassay and gas chromatography–mass spectrometry. From Ref. [157], with permission.

6.2.6. Sample pretreatment before ELISA measurements

Methods for sample extraction prior to IA analysis have been discussed [167,168]. First, approaches that emphasize speed and minimal steps are desirable to keep costs down. For solid samples and more complex matrices such as soil or foodstuffs, some sample preparation is generally needed. Classical extraction methods can be used. The combination of supercritical fluid extraction or microwave-assisted extraction with ELISA has been described for the determination of pesticides and PCBs in soils and sediments [169,170]. For soils or sediments, the extract is usually diluted before the ELISA measurement, so that the matrix effect is greatly reduced. One relevant example deals with the control of atrazine residue in soil after the atrazine ban in

TABLE 6.8

CONCENTRATION ($\mu\text{g/l}$) OF ATRAZINE AND SIMAZINE IN 1 l OF PREVIOUSLY FREEZE-DRIED WATER SAMPLE USING DIFFERENT TECHNIQUES ($n = 6$) (FROM REF. [148], WITH PERMISSION)

Technique	Batch A		Batch B	
	Atrazine	Simazine	Atrazine	Simazine
Direct ELISA	1.63 ± 0.10	–	1.68 ± 0.15	–
LLE-Florisil-ELISA	0.78 ± 0.08	–	1.39 ± 0.15	–
LLE-Florisil GC-NPD	0.62 ± 0.04	1.89 ± 0.14	1.39 ± 0.07	0.88 ± 0.09
% Recovery	126		100	

Germany [171]. The comparison of results with HPLC was excellent (slope of 1.034) when the extracts were diluted 1:500, whereas a small bias was observed (slope of 0.858) when the same extracts were diluted 1:150.

Usually, water samples are analyzed without sample pretreatment, since the typical environmental concentrations are in the range 0.1–10 $\mu\text{g/l}$: many examples have already been mentioned. Sample pretreatment is required when the expected concentrations are below or close to the limit of quantification, or when there is a strong matrix effect because of humic substances, for example. In that case, solid-phase extraction using disposable cartridges is well suited, the methanol desorption solution being diluted in buffer.

Aga and Thurman [172] have applied a pretreatment as described above for the determination of atrazine in surface water samples from six lakes in the Isles Royal National Park (Mi, USA) using SPE-ELISA and GC-MS. The determined atrazine concentrations were in the range 2–20 ng/l, so an SPE step was required before ELISA. The same workers have also compared two atrazine ELISA kits. The correlation coefficient between magnetic particle-based ELISA and GC-MS was 0.96 with a regression line slope of 0.89, and the correlation coefficient between microtitre-plate-based ELISA and GC-MS was 0.93, with a regression line slope of 0.95 [172]. Although one can expect a low concentration of de-ethylatrazine in these samples, the regression slope was higher with the microtitre plate-based assay which shows less cross-reactivity with this metabolite.

Lucas et al. [82] analyzed 75 well water samples for atrazine in a blind fashion. They found a good correlation with the GC method, with only one false negative and no false positive using ELISA. They applied a rapid SPE sequence using a C₁₈ cartridge (100 ml sample) in order to reduce the detection limits and to have data in the middle of the working range of their IAs. The objective of this sample pretreatment was also to increase confidence in the data by eliminating potential interferences such as pH extremes, high ionic strengths, or potentially immunoreactive metabolites. It took 1 day for one trained analyst to complete the 75 well water samples,

from extraction with the SPE C₁₈ cartridges, to generation, and to the final atrazine concentration data from ELISA. The extra time and effort spent in using SPE permitted direct analysis by GC for confirmation, using the ethyl acetate extract.

The coupling of SPE-ELISA was also applied to the simultaneous determination of alachlor and the ethanesulfonic acid (ESA) metabolite [154]. The anti-alachlor antibody cross-reacts with ESA, which produced false positive detections of alachlor in water samples by the ELISA kit. Alachlor and ESA were first isolated from water by SPE on a C₁₈ cartridge and then eluted sequentially with ethyl acetate and methanol. Alachlor is soluble in ethyl acetate while the anionic ESA is not. Thus, ESA remains on the cartridge and is eluted later by methanol. The combination of SPE with ELISA effectively separates and quantifies both alachlor and ESA, using the same antibody for the two ELISA methods, with detection limits of 0.01 µg/l for alachlor and 0.05 µg/l for ESA. Table 6.9 shows the analysis of ground water samples using the analytical procedure described above. The agreement between GC-MS and the SPE-ELISA determination for alachlor is good. The results for ESA concentration were confirmed by HPLC, and have shown that the values measured by the kit were mainly due to ESA and not to alachlor, thus explaining the false positive values.

6.2.7. Quality assurance and guidelines for validation and use

The acceptance of immunoassays is dependent upon the demonstration of quality and validity compared to more traditional methods. We have already pointed out that

TABLE 6.9
ANALYSIS OF GROUND WATER SAMPLES FOR ALACHLOR AND ESA BY ELISA, SPE-ELISA AND GC-MS (FROM REF. [154], WITH PERMISSION)

Sample	Alachlor concentration (µg/l)			ESA concentration (µg/l)
	Direct analysis by ELISA	GC-MS	SPE-ELISA	SPE-ELISA
1	0.6	0.45	0.50	0.06
2	0.16	<0.05	0.04	0.83
3	0.92	<0.05	0.05	2.18
4	0.33	<0.05	0.03	1.65
5	1.83	0.07	0.10	4.80
6	1.10	0.21	0.39	1.88
7	0.22	<0.05	0.04	1.10
8	8.60	1.50	1.26	3.50
9	0.22	0.06	0.09	0.91
10	2.85	0.52	0.61	9.32
11	1.79	0.36	0.33	4.29
12	4.63	2.95	2.78	5.41

both the strength and the weakness of immunoassays must be considered in order to use them effectively and efficiently. Depending on the type of environmental analyses and on the target pesticides, IAs can provide good screening methods, but can also provide quantitative measurements. Critical evaluation of the analytical requirements is needed before determining how best to use immunoassays. Confirmation of positive samples must be planned, according to the analytical requirements. If many samples are to be analyzed but only a few contaminated samples are expected, the use of ELISAs for screening, with re-analysis of the positive samples by chromatographic methods may be a good use of IAs. The definition of “positive sample” may be different, depending on whether the survey is made for regulatory purposes or to study the transport or fate of a target pesticide. If many samples are likely to be contaminated, as in studies for field dissipation or for pesticide registration studies, the confirmation of positive detections will be expensive and will diminish the advantages of using immunoassays. In such conditions, it is worthwhile having analytical conditions so that the ELISAs should be quantitative.

In this section, specific guidelines for the validation and use of immunochemical environmental methods are presented.

6.2.7.1. Quality standard for immunoassay kits

Immunoassays have not yet been extensively characterized. Several agencies in the USA and in Europe (EPA, AOAC, US Analytical Environmental Immunochemical Consortium or AEIC, German Immunoassay Study Group) are involved in their evaluation and the proposal of guidelines. Their aim is to help assure that high quality performance and the appropriate interpretation of results are obtained from immunoassay kits used for a variety of applications by operators with varying degrees of experience. As we have mentioned, commercial immunoassay kits are now supplied with information sheets that describe various items such as the kit contents, test procedures, and expected performance characteristics. This is one result of the development of recommended standards by the AEIC [173]. The AEIC is also developing standardized definitions for terms that are frequently used to describe immunoassay kits and their associated performance characteristics.

The initial recommended standards include establishing a standardized package insert, the source of immunoassay kit calibrators and samples for control, and quality control guidelines for use in monitoring kit performance. A variety of quality control information is given with the results obtained by using the ELISA kit, i.e., dose-response curve, 50% inhibition concentration, range of quantitation, precision of replicate standards, and level of range expected for the negative control. However, quality information about data generated from field samples is not provided and its provision is a priority; this should include guidelines for use and validation.

6.2.7.2. Guidelines for validation

Validation first requires the demonstration that data generated by the immuno-

assays are comparable to, or better than, data generated by traditional analytical methods. However, the validation guidelines depend on whether the immunoassays are used to complement the traditional analytical methods or to replace them. For quantitative methods, confirmation of the limits of quantitation, delineation of the quantitative range, evaluation of interferences, and estimation of the accuracy and precision with field samples, are required. When a sample pretreatment (extraction, clean-up, etc.) is required, it should be included in the method validation and described in the written procedure. Information to be included in written immunological analytical methods can be found in Ref. [174], which describes the guidelines for using immunoassays in support of pesticide registration.

6.2.7.3. Appropriate use of immunoassays for environmental applications and confirmation of results

An appropriate use of immunoassays is in first-screening and, when possible, quantitation, in environmental fate and residue studies, because there are many samples to analyze. Conventional methods cannot compete for speed and cost of analysis. Immunoassays can also be selected because analyses can be performed in the field. Other examples of appropriate use exist, when they provide sensitivity not attainable by other methods or when traditional methods are too cumbersome at the required limit of detection. Their use is inappropriate when there is only a small number of samples, when the matrix effects are too important, or when the purpose of the study is to identify transformation products. They cannot be used for quantitative purpose when cross-reactants are present in the samples.

The need to confirm positive and negative samples using other analytical methods depends mainly on the type of study and on the intended use of the data. For enforcement applications, such as water monitoring, ideally all positive samples should be confirmed. However, when most detections are very small, the expense resulting from residue verification would not be justified and confirmatory analyses should be performed on detects of regulatory significance. The regulatory cut-off level should be determined depending on the compounds and the regulatory levels. To ensure that false negatives are not occurring, a fraction of the negative samples should be re-analyzed by another method.

Immunochemical methods are particularly well adapted to environmental fate studies, i.e., of aquatic and terrestrial field dissipation, ground water, and run-off studies. In some cases, only one compound is applied and interferences from other pesticides are not expected to occur. Depending on the persistence properties, positive samples may be expected for quantitative data. Before designing a confirmation scheme it is important to know the potential for interference by cross-reacting transformation products. The Environmental Agency Office of Pesticide Products (EPA/OPP) has proposed a scheme for confirmation of residues in water samples for field studies in which immunoassays are used to screen for positives [173,174]. A statistically representative number of the positive and negative samples should be re-

analyzed using a non-immunochemical method for confirmation. The identity of the analytes should also be confirmed in a subset of samples containing residues in concentrations of toxicological or environmental concern. The frequency of confirmation should be reduced as the monitoring proceeds, assuming good agreement between the methods, and where the term “agreement” has previously been established according to proper evaluation criteria.

An interesting field dissipation study was recently described for the disappearance of aerially applied fenitrothion in rice-crop waters in Spain [175]. For monitoring the fenitrothion residues in water, two different analytical techniques were used: an ELISA kit, and an automated on-line solid-phase extraction (Prospekt) followed by liquid chromatography with diode array detection or MS detection for unequivocal confirmation. Since there is no ELISA kit sensitive enough for measuring fenitrothion, the ELISA kit corresponded to parathion-ethyl. It was shown that this kit cross-reacts with fenitrothion with a detection limit of $1.3 \mu\text{g/l}$. This ELISA could be used because parathion-ethyl is not used on rice and has not been used for at least the last 20 years in the area of interest. It was also verified that the kit did not respond to fenitrooxon, the major metabolite of fenitrothion. Results from ELISA were confirmed by results from SPE-LC-MS. This is a typical example where ELISA can be appropriate for field study.

6.2.8. Other formats of immunoassays

There have been several attempts to simplify the ELISA's formats, such as reducing the number of washing steps, avoiding the separation between the free and bound analyte, or accelerating the equilibrium step. Field formats based on the use of strips have also been described. Other attempts have been made to automate IAs on site with flow injection techniques.

6.2.8.1. Polarization fluoro-immunoassays

Polarization fluoro-immunoassay (PFIA) is a homogeneous immunochemical method, so does not require washing or separation of the free and bound analyte. It is a competition method, based on the detection of the difference of fluorescence polarization between a small fluorescent-labelled antigen and its immuno-complex with a specific antibody. It depends on the difference in the signal given by a relatively small fluorescing-labelled hapten, when it is in the free form, as compared with the much higher polarization values when it has been bound to its specific antibody. The polarization or fluorescence is determined by exciting the mixture of antibody, sample, and tracer with vertically polarized light and measuring the intensity of both the vertically and horizontally polarized components of the emitted fluorescence.

PFIAs are used in clinical chemistry because of their simplicity, precision and possible automation. The first application to pesticides was developed by Colbert and Coxon for the determination of paraquat in serum samples [176]. PFIAs have

recently been optimized for the rapid detection of 2,4-di- and 2,4,5-trichlorophenoxyacetic acids (2,4-D and 2,4,5-T) and of simazine and atrazine [177]. Detection limits of 100 $\mu\text{g/l}$ for 2,4-D and 5 $\mu\text{g/l}$ for simazine are reported in this study.

6.2.8.2. Flow-injection immunoassays

On-site, automated flow-injection monitors represent a low cost option for obtaining continuous and quantitative data on dissolved aquatic chemical parameters [178]. Much research, recently reviewed [179], is being devoted to the automation of immunoassays through the use of continuous-flow systems.

In the simplest form of the method – homogeneous flow-injection immunoassays (FIIA) – no separation of bound and free species is required. The sample is injected into the flow stream containing reagents. Usually, a labelled antibody present in the flow stream produces a signal which changes when the antibody–antigen complex is formed, owing to the quenching of a fluorescent label or the inhibition of an enzyme label by the binding reaction.

Heterogeneous FIAs require the separation of bound and free labels. The antibodies are usually immobilized onto a solid support contained in an immunoreactor column. Samples and reagents are injected into the flow system. The analyte to be determined in the sample, and the corresponding enzyme-labelled analyte, are allowed to compete for a limited number of antibodies. The system works in the sequential saturation analysis mode, which means that the analyte and the enzyme tracer are sequentially incubated with the antibodies. Then, the substrate for the enzyme tracer is pumped and the signal of the enzyme-generated product is measured downstream. Fluorescence is commonly used as signal because of its sensitivity using fluorimeter flow-through cells. The fluorescence intensity is measured using the peak height, giving rise to quantitative data. Regeneration is necessary and is obtained by washing with specific buffers. Such a system was used for the detection of atrazine with possible regeneration for up to 60 runs [180–182]. Recently, the optimization of a similar FIIA system was presented, using an affinity column with protein G immobilized onto glass beads with the antibodies being pumped through this column and briefly incubated [183]. This has the advantage of providing a more universal system because the authors have noticed that each antibody–antigen system showed different stability and regeneration features. With this system, the independence of the regeneration of the antibody system is gained because this regeneration is made through the protein G-antibody site. It was then possible to regenerate the protein G column more than 200 times, with only a minor reduction in activity, and with good reproducibility of regeneration. High antibody dilutions (1:20 000 to 1:50 000) are used, which represents a very low antibody consumption. Research is currently done so this FIIA system can work alone for up to 2 weeks at least.

6.2.8.3. Dipstick immunoassays

Immunoassays in a dipstick format have been studied [181] and should certainly

become available commercially in the near future for the environmental analysis of pesticides. Their main advantage is in easy field use, just by dipping the strips into the water, with no sample preparation. The dipstick techniques involve first the competitive immuno-reaction and then the colour development. Test strips are usually prepared by applying a coating of primary antibody and then incubating with the specific antibody. Once the strip is prepared, the immunoassay dipstick is dipped into the solution and the enzyme tracer, and then incubated during a certain time. The enzyme substrate is added and incubated if necessary. The dipstick is then removed from the solution and quantitative measurements can be obtained photometrically. Another strip format has been described, based on immuno-migration using liposomes, and is described below.

6.2.8.4. *Liposome-amplified immunoanalysis*

The advantages have been demonstrated by Durst et al. [184,185] of liposome-encapsulated markers which acts as signal enhancers of the competitive binding reaction of small molecule immunoassays, instead of using enzymatically produced colours. Liposomes provide instantaneous, rather than time-dependent, enhancement and offer considerable potential for both automated and field assays. Single-use field immuno-migration strips have been applied for the determination of alachlor and PCBs [186–188].

Liposomes are bilayered vesicles that are formed spontaneously when lipids are dispersed in water. During formation they encapsulate a portion of the aqueous solution in which they are dispersed, and if this solution contains a marker molecule, such as a dye, this will be present in the aqueous core of the liposome. If the analyte of interest is conjugated to a lipid this also can be incorporated into the liposome surface. Figure 6.20 shows the principle of a competitive liposome immunoassay. The tagged liposomes and the sample containing the analyte are passed over a solid surface onto which the antibodies have been immobilized. Competition then occurs between the free analyte molecules and those which are conjugated to the liposomes. The number of liposomes that bind the antibodies is inversely proportional to the amount of free analyte in the sample. Unbound liposomes move out from the antibody region and can be measured by an appropriate downstream detector. Alternatively, the bound liposomes can be measured in situ, or a detergent can be added into a flowing stream to release the marker which is then measured downstream. The use of liposomes instead of the more usual enzyme-produced markers has several advantages. The lipid composition can vary to provide the liposomes with different characteristics, and almost any water-soluble marker can be encapsulated, giving rise to several possibilities for detection. The liposome size, and the surface concentration of an analyte tag can be varied and controlled accurately. Enhancement is instantaneous, removing the requirement for a timed enzymatic incubation step, and the whole process can be automated [187].

Flow injection liposome immunoassays have been described. The sample and

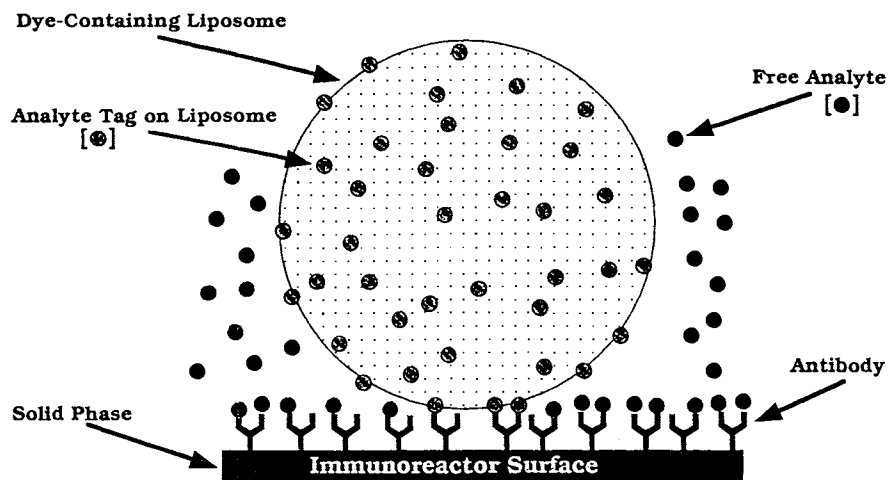


Fig. 6.20. Description of the competitive-binding reaction between liposomes and analyte molecules for antibody sites on an immunoreactor solid surface. From Ref. [187].

liposomes in the carrier are passed through the same, reusable immunoreactor column which contains an inert support with the antibodies conjugated to it. Competitive binding occurs, with a higher level of samples causing fewer liposomes to bind to the column. Unbound liposomes and sample pass through the column and go to waste. After washing, detergent is passed through to lyse the liposome and release the marker which is detected downstream and quantitated. The system is reused after regeneration of the immunocolumn. With the simple design shown in

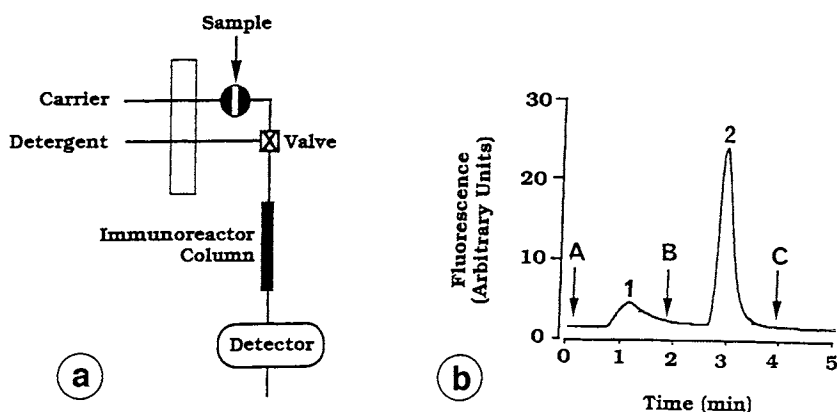


Fig. 6.21. (a) Diagram of a simple flow-injection system and (b) signal obtained in a typical flow-injection liposome immunoassay. (A) corresponds to the addition of sample into the carrier stream, (B) to the addition of detergent, and (C) to the addition of the carrier for column regeneration during 1 min. See text for peak characteristics. Adapted from Ref. [188].

Fig 6.21a, and using anti-alachlor antibodies in the immunoreactor column and alachlor-tagged liposomes containing sulforhodamine B, a typical signal is obtained as in Fig. 6.21b using fluorescence detection. The small initial peak (1) corresponds to the quenched fluorescence of intact liposomes that do not bind to the column whereas peak (2) is produced by the released dye and is used for quantitation. The total assay time can be as short as 5 min, and it is possible to quantitate alachlor down to the 5–10 $\mu\text{g/l}$ level.

Field immunomigration strip assays have been developed by the same research group as above [186–188]. In a liposome immunomigration competition assay shown in Fig. 6.22, liposomes and analyte molecules are allowed to migrate up a double-zone strip of adsorbent material on which anti-analyte antibodies (competition zone) and anti-biotin (capture and measurement zone) have been immobilized. The competition occurs in the antibody zone; the amount of liposome bound is quantified by the amount of colour from the encapsulated (B), and is inversely proportional to the amount of analyte in solution. A second zone, containing anti-biotin to bind the liposome, collects all the liposomes that do not bind to the antibody zone, and the amount of colour in this zone is directly proportional to the amount of analyte in the sample. Therefore, quantitation in either zone is possible, with the use of the second zone being more “intuitive and preferred” according to the authors [187]. This assay has the potential for rapid field-screening and is simple.

A prototype assay based on the preceding principles was developed for alachlor determination. A protein-binding membrane (a porous nitrocellulose membrane) with a plastic backing was employed to provide rigidity. The assay is performed by dispensing one drop of the sample or control solution and one drop of a concentrated

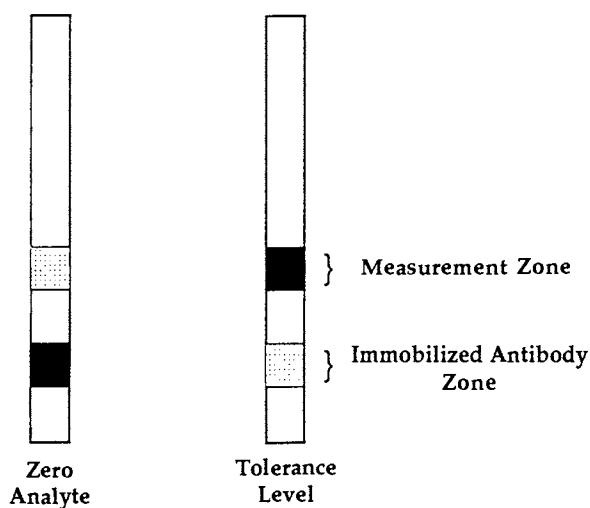


Fig. 6. 22. Diagram of a liposome immuno-migration competition assay. The grey-scale density of zones indicates the liposome concentration. From Ref. [187].

buffer into a glass test tube, mixing the contents, and adding one drop of a liposome solution. After mild shaking, the test strip is inserted into the tube and is left there until the solution front reaches the end of the strip (about 8 min). Then the strip is removed and air-dried and the colour intensity of the antibody zone and the anti-biotin zone are estimated, either optically or by scanning densitometry. Dose-response data were obtained by scanning densitometry of strips run in the presence of various concentrations of alachlor. The responses in both zones varied logarithmically with alachlor concentration, and both were estimated to detect $10\text{ }\mu\text{g/l}$ of alachlor.

A second liposome immunoaggregation assay has been developed which uses plastic-backed nitrocellulose strip similar to that above, but with only a liposome capture zone applied. The competition for the antibody, between analyte-tagged liposomes and free analytes, is allowed to occur in a test tube for a few minutes, rather than in the antibody zone applied to the strip. As a single antibody can react with two liposomes, repetition of this step leads to the formation of large liposome-antibody aggregates which do not migrate up the strip but adhere to its nitrocellulose surface at the meniscus of the incubation solution. This aggregation formation is inhibited by the occurrence of free analytes and thus the amount of aggregation is inversely proportional to the amount of analyte.

6.2.9. Conclusions

Immunoassay techniques provide a simple, powerful and inexpensive screening method with enormous potential, which includes the generation of quantitative data. They are gaining acceptance and the confidence of analytical chemists. They are competing successfully with traditional analytical methods because they are now evaluated by the same criteria according to well-defined quality assurance plans. However, users must know and recognize their limitations. In particular, they must be aware of the data interpretation and know that, depending on the selected application, some immunoassays can be quantitative whereas others cannot. Given their widespread adoption, and the ease with which these assays are performed, many people, manufacturers, academic and industrial researchers and end-users, should be concerned with correct data generation and interpretation. The development of guidelines will promote consistent validation and data reporting, and applications will certainly help the wider acceptance of this technology.

6.3. IMMUNOCHEMICAL SAMPLE PREPARATION METHODS

The increasing development of antibodies tailored for immunoassays has induced their use in other fields, and especially in the sample-preparation area. Immunochemical sample preparation methods also use the antigen-antibody reaction for the selective extraction of an analyte or a group of structurally related analytes from

complex environmental matrices. Antibodies are covalently bonded onto an appropriate sorbent to form a so-called immunosorbent (IS), to be packed into a solid-phase extraction cartridge or precolumn. In contrast to IAs, the ISs provide individual quantification of each analyte because, once the compounds have been extracted onto the IS, they are desorbed, separated, and analyzed. In this section, we describe the development and the expected properties of immunosorbents in relation to the antibody characteristics

Immunochemistry was introduced a long time ago for analysis and/or sample pretreatment in the medical and biological fields. Its introduction into the environmental field is, however, relatively recent because of the difficulty in making selective antibodies for small molecules. In the medical field, ISs have often been developed for the analysis of a single analyte and its metabolites which are structurally related to the parent molecule. Immunosorbents are also currently being developed by several agricultural chemical companies for use in studies to support pesticide registration for some target pesticides and degradation products [189,190].

The first commercial ISs used in environmental analysis were introduced for the clean-up of samples prior to the determination of aflatoxins [191,192]. Other ISs have been described in the literature for the analysis of single pesticides such as carbendazim, chlortoluron, atrazine or terbuthylazine [193–196]. Because of the unavoidable cross-reactivity of antibodies against atrazine, ISs were also developed for trapping atrazine and its major degradation products [197]. Our own group took advantage of the cross-reactivities of antibodies to develop ISs that were able to extract selectively a whole group of structurally related compounds such as the triazine and phenylurea pesticides [37,198–202]. An IS obtained by bonding anti-chlortoluron antibodies onto silica was shown to trap ten phenylureas, with good recoveries for most of them. The high selectivity of these ISs has been illustrated in the handling of highly contaminated surface waters and also plants and foodstuffs. For surface water samples, the extraction, concentration and clean-up are performed at the same time and their chromatograms were as clean as those obtained in LC-grade water, thus allowing an easier quantification and identification at the 0.1 μl level (see Figs. 4.22 and 5.9).

Class-selective trapping is now recognized as a key feature of ISs to be tailored for environmental analyses of pesticides. There is also an interest in developing IS for single analytes such as aminotriazole which are particularly difficult to analyze at trace levels because of a lack of available extraction methods from water.

6.3.1. Synthesis of the immunosorbents

Immunosorbents are obtained by covalently binding antibodies onto appropriate sorbents. The selected sorbent should have large pores, because antibodies are large molecules, be hydrophilic in order to avoid any non-specific interactions, and be pressure resistant so as to be used in on-line techniques. The third requirement is not

obligatory for off-line disposable cartridges, and agarose is often used for antibody immobilization. Only silica-based sorbents meet the three requirements, and in order to reduce the preparation step it is faster to use commercial silicas already modified by appropriate functional groups. Our first ISs were made from silica containing active aldehyde groups and a pore size of 300 nm [37]. However, the results showed a rather low capacity, and a silica-based sorbent with a higher specific area but lower pore size (50 nm) is now selected, although there is a compromise between large pore sizes and high specific areas.

Many workers have attempted to use hydrophilic polymers because they allow an oriented bonding of the antibodies and have higher capacities. The bonding procedure using silica-based sorbents gives rise to antibodies grafted onto the surface in a random way, so some antibodies are not well orientated towards the analytes. However, hydrophilic polymers all contain π -bonds which automatically give rise to non-specific hydrophobic interactions and reduce dramatically the selectivity of the IS, which is the primary aim of the sample preparation methods.

6.3.2. Characterization of the class-specific immunosorbents

Group extraction is possible if the antibody bonded to the sorbent can cross-react with structurally related compounds within the group of pesticides. This largely depends on the cross-reactivity developed by the antibodies and on the structure of the compounds within the group. Moreover, the possibility exists for bonding two or three different antibodies in order to cover the extraction of the whole group. The cross-reactivity which is determined with immunoassays is different from that which exists with an immunosorbent using the same antibodies. In ELISAs, the cross-reactivity measured with the kit is the result of both the cross-reactivity of the antibody and the competition reaction with the labelled analyte, whereas with an IS, there is no competition with a labelled antigen. All of the analytes compete for the antigen binding sites and, depending on their affinity, some compounds are more retained by the IS than other ones. Once again, the hapten design is important. For making a class-specific IS, one has to obtain antibodies which show as much cross-reactivity as possible. Hage et al. have bonded a monoclonal antibody onto silica for trapping atrazine and its major metabolites de-ethyl-, de-isopropyl- and hydroxy-atrazine [197]. Using an antibody raised against imazethapyr, Wong et al. [189] were able to trap other imidazolinone compounds and metabolites which retain the imidazolinone ring structure. We have shown that when the immunization time in rabbits was increased the cross-reactivity for the related compounds increased [37]. Table 6.10 shows the potential for class-specific ISs obtained for phenylureas when bonding polyclonal anti-isoproturon or anti-chlortoluron antibodies and for triazines when bonding anti-atrazine or anti-simazine antibodies [37,198,199,202]. For the group of phenylureas it was difficult to predict that the anti-isoproturon IS should be class-specific because its structure is slightly different from those of other phenylureas.

This was easier for anti-chlortoluron antibodies, and this IS shows good recoveries for almost all the compounds except fenuron which has no substituent on the phenyl ring and fluometuron. For triazines, it was predicted that the anti-atrazine antibody should be unable to cross-react with de-isopropylatrazine, and that was experimentally verified, whereas the anti-simazine cross-reacts with this analyte. Therefore, if one wants to trap all the triazine class, including metabolites, it is appropriate to mix the two IS obtained with anti-atrazine and anti-simazine antibodies in a single cartridge. It is worthy of note that the extraction procedure is similar for the two classes (i.e., conditioning with a buffer, sample application, and desorption with a few millilitres of methanol–water mixture, 70:30, v/v) so that multi-residue extraction can be obtained for these two classes using a single extraction cartridge packed with a mixed bed of anti-triazine and anti-phenylurea ISs.

The results in Table 6.10 were measured with a mixture of compounds, each one at the same concentration. We can expect the lowest recoveries to be obtained for compounds having the lowest affinity for the antibodies, but low recoveries can also be explained by overloading of the capacity. For quantitative purposes, it is important to know how an IS works and the relationship that exists between recovery, capacity and affinity order. In other words, since there is a competition reaction among the various analytes in the group, the question is whether the recoveries depend on the number of related analytes in the sample and on their respective concentrations.

TABLE 6.10
POTENTIAL FOR CLASS-SPECIFIC IMMUNOSORBENTS AS MEASURED BY RECOVERIES
OBTAINED FOR MIXTURES OF PHENYLUREAS OR TRIAZINES

Analytes	Extraction recovery (%)		Analytes	Extraction recovery (%)	
	Anti-iso-proturon IS ^a	Anti-chlor-toluron IS ^b		Anti-atrazine IS ^c	Anti-simazine IS ^d
Metoxuron	21	80	De-isopropylatrazine	<5	56
Monuron	98	78	Hydroxyatrazine	60	<5
Chlortoluron	95	95	De-ethylatrazine	98	30
Isoproturon	99	90	Simazine	99	93
Difenoxyuron	37	17	Cyanazine	91	74
Buturon	64	62	Simetryn	63	17
Linuron	61	85	Atrazine	99	88
Chlorbromuron	95	102	Prometon	65	<5
Diflubenzuron	101	76	Sebutylazine	88	91
			Propazine	101	57
			Terbutylazine	98	85

Mean values of three experiments, average RSD values in the range 3–9%. Recoveries measured by percolation of:

^{a,b}50 ml of water spiked with 0.5 µg/l of each analyte.

^{c,d}25 ml of water spiked with 3 µg/l of each analyte.

6.3.2.1. Evidence for different affinities within the group

Antibodies, which are group specific, show a very strong affinity for the analyte-antigen. A consequence is that desorption can only be obtained with conditions which are unusually strong for antibodies. With the three IS used in Table 6.10, it was necessary to use 2–3 ml of a buffer solution containing 70% methanol to desorb all the analytes and especially the analyte-antigen.

Analytes within a group have different affinity orders for the antibodies. This was shown by using a step desorption from the anti-isoproturon IS [37]. After loading the IS with a 50 ml water sample containing 13 phenylureas, the desorption was achieved with 3 ml fractions of formic acid (0.2%, v/v) containing increasing amounts of methanol. Each fraction was analyzed separately. It was shown that fenuron, metabenzthiazuron and fluometuron were not retained at all, that seven other compounds were in various proportions in the first two fractions containing pure formic acid and formic acid with 20% methanol, whereas monuron, chlortoluron and neburon were mainly in the third fraction containing 40% methanol. The antigen isoproturon was 18% desorbed in the fifth fraction containing 60% methanol, and the remaining 82% was in the sixth fraction, containing 70% methanol.

Another important feature of the IS is their high stability. ISs can easily be regenerated using a PBS solution. It was verified that after 50 runs, the loss in capacity was less than 10% [198,199]. The ISs can be submitted to a high proportion of organic solvents, which destroy the antigen–antibody interaction, certainly by deformation of the conformation of the protein, but in a reversible way, because antibodies have been stabilized by the covalent binding to the silica.

6.3.2.2. Capacity

In many studies, the IS capacity is determined using the pesticide antigen. However, owing to the affinity chromatography process, this capacity cannot be transposed to the other compounds within the group, as shown below.

6.3.2.2.1. Capacities measured with the pesticide-antigen. The capacity is usually defined as the total number of immobilized active antibodies. Antibodies are contained in the G-type immunoglobulin (IgG) fraction which has been isolated from the serum of the immunized animals. It is not simple to measure the concentration of active antibodies (i.e., antibodies specific for the targeted pesticide) in this IgG fraction, and it is commonly found in the literature that the IgG fraction contains about 15% active antibodies. During the covalent bonding of the antibodies, only the amount of immobilized IgG can be estimated, and this is shown in Table 6.11 for three ISs obtained on a silica-based sorbent using three different antibodies. The capacity was first measured using the pesticide antigen which had been used for developing the antibodies, since the antibody affinity is expected to be highest for this compound. Water samples were spiked with increasing concentrations of pesticide antigen, up to 150 µg/l. The curve representing the amount of atrazine

TABLE 6.11
CAPACITY OF THREE IMMUNOSORBENTS MEASURED BY THE PESTICIDE ANTIGEN

Antibodies	Immunosorbent		
	IgG ^a	Capacity ^b	Capacity ^c
Isoproturon	35 ± 2	19.3 ± 0.6	0.55 ± 0.04
Atrazine	47 ± 2	2.5 ± 0.2	0.053 ± 0.005
Simazine	54 ± 2	0.80 ± 0.05	0.015 ± 0.002

Silica with 50 nm pore sizes.

^aAmount of IgG in mg/g of silica-based sorbent.

^bCapacity in $\mu\text{g/g}$ of IS.

^cCapacity in $\mu\text{g/mg}$ of IgG. Capacity measurements with increasing concentrations (5–150 $\mu\text{g/l}$) of analytes in 50 ml of LC-grade water for isoproturon, and in 25 ml for atrazine and simazine.

adsorbed on the anti-atrazine IS as a function of the atrazine concentration in the percolated sample has a Langmuir shape with a linear part and a plateau where the capacity can be experimentally estimated. The linear part of the adsorption isotherm depends on the ISs and were found to be in the 0–80 $\mu\text{g/l}$ range for isoproturon, 0–20 $\mu\text{g/l}$ for atrazine, and 0–7 $\mu\text{g/l}$ for simazine. The capacities, as represented in Table 6.11, are usually estimated on the plateau for the highest concentrations.

The capacity of an IS depends greatly on the concentration of active antibodies in the IgG fraction. It is obvious that the IgG fractions were more concentrated in anti-isoproturon antibodies than in anti-simazine or anti-atrazine antibodies. This concentration was found to be independent of the rabbits used for immunization, with a similar period of immunization [37]. These results also indicate that the first condition for reproducibility of ISs is to have reproducible batches of antibodies, which can only be obtained by making monoclonal antibodies. Current studies indicate that ISs prepared from monoclonal antibodies for phenylureas or triazines have similar properties for group extraction, and that monoclonal antibodies show similar cross-reactivities to polyclonal antibodies, which can be explained easily by the fact that the molecules of interest are small [203]. Since polyclonal antibodies are easier to produce and much cheaper, optimizing the characteristics of ISs with polyclonal antibodies before the selection of monoclonal antibodies is a very convenient and useful approach.

6.3.2.2.2. Capacities measured with a group of related compounds. The competition process between the compounds in a group is illustrated by the measurements of capacities using water samples spiked with a mixture of ten phenylureas (representing almost all the phenylureas on the market) for the anti-isoproturon IS, and with a mixture of seven triazines for the anti-atrazine and anti-simazine ISs. The compounds in each mixture were at the same concentration, which varied in the range 1–100 $\mu\text{g/l}$. For isoproturon, the same curve was obtained, but

with a lower linear part $0\text{--}50\text{ }\mu\text{g/l}$ instead of $0\text{--}80\text{ }\mu\text{g/l}$, when it is alone in the sample, and a lower value for the plateau. For the other phenylureas, the linear part was much narrower, in the range $0\text{--}10\text{ }\mu\text{g/l}$ for monuron, chlortoluron and difluzbenzuron, and in the range $0\text{--}5\text{ }\mu\text{g/l}$ for all other phenylureas. Table 6.12 reports the maximal amounts which have been obtained for each individual curve for the handling of water samples spiked with $100\text{ }\mu\text{g/l}$ of each phenylurea. One can see the lower capacity thus measured for isoproturon in a mixture, $11.8\text{ }\mu\text{g/g}$ IS, instead of $19.3\text{ }\mu\text{g/g}$ IS when it is on its own. However, the isoproturon capacity is still much higher than the capacities measured for the other related compounds, which depend on the affinity of the antibodies for the compounds. A remarkable result is that the sum of the capacities for the related compounds, and for isoproturon in a mixture is close to the capacity measured for isoproturon alone. Similar results were obtained for the two triazine ISs. These results clearly indicate the competition process for the antibody recognition sites. Therefore, the capacity measured for each analyte using a mixture of compounds depends strongly on the competition process, i.e., on the number of compounds and their respective concentrations in the sample.

6.3.2.3. Limitation of recoveries by capacity and/or affinity of compounds

In a solid-phase extraction process, an incomplete recovery is explained by analyte breakthrough which can be the result either of an insufficient retention or overloading of the capacity of the sorbent (see Chapter 4). With C_{18} silicas the capacity is high and when compounds are present at the $\mu\text{g/l}$ level, breakthrough mainly occurs because of insufficient retention. The capacities of the ISs are sufficient for the analyte-antigen, but this condition is not straightforward for the related analytes, espe-

TABLE 6.12
CAPACITY MEASURED BY THE CORRESPONDING AMOUNT OF EACH PHENYLUREA
ADSORBED ON THE ANTI-ISOPROTURON IMMUNOSORBENTS

Analyte	Amount adsorbed in $\mu\text{g/g}$ IS
Fenuron	0.29 ± 0.02
Metoxuron	0.40 ± 0.05
Monuron	0.49 ± 0.05
Chlortoluron	0.58 ± 0.05
Isoproturon	11.8 ± 0.2
Difenoxuron	0.95 ± 0.06
Buturon	0.52 ± 0.05
Linuron	0.72 ± 0.07
Chlorbromuron	1.21 ± 0.08
Difluzbenzuron	0.51 ± 0.04

Percolation of 50 ml of water spiked with a mixture containing $100\text{ }\mu\text{g/l}$ of each analyte; mean values from two measurements.

cially those having a low affinity for the antibodies. Therefore, an incomplete recovery can be the result of a low capacity or/and a low retention.

Calibration curves constructed for a given volume of water sample containing increasing amounts of all the related analytes were shown to reflect the adsorption isotherms, with a linear range followed by a plateau [202]. Therefore, the recovery is expected to remain constant in this linear range and its value will depend on the sample volume. If the volume of sample used for constructing the calibration curves is below the breakthrough volume, a theoretical recovery of 100% is to be expected. For the concentration range corresponding to the plateau, the capacity is exceeded, and increasing the concentration of the percolated solution will reduce the recovery since the amount adsorbed on the IS no longer increases. Because the breakthrough volume depends on the antibody affinity for the analytes, there is a close relationship between the recoveries and the analyte capacity and affinity.

Quantitative analysis can be made only if recoveries are constant over the whole linear calibration range and if the calibration curves do not depend on the competition process. In other words, the calibration curves and recoveries should be similar, independent of whether the antigen-analyte or any other related analyte is on its own or in a mixture of related compounds. This is the situation that occurs in an unknown sample, because one does not know the number of compounds present in the sample or their respective concentrations. Recent studies have confirmed that recoveries did not depend on the number of analytes present in the sample, provided the capacity was not overloaded. This was verified for metoxuron, which showed % recoveries of (61 ± 4) when in admixture with seven other phenylureas at the $1 \mu\text{g/l}$ level, and (55 ± 3) at the $2 \mu\text{g/l}$ level. With the same sample volume (25 ml) and amount of IS, the recovery was 57% in water samples spiked with metoxuron alone, at two concentration levels (1 and $2 \mu\text{g/l}$). Therefore, it is interesting to have a maximum capacity for the IS because the linear calibration range of related compounds increases with capacity [202].

When 220 mg of anti-isoproturon IS was used, with a sample volume of 50 ml, typical linear ranges were 0–10 $\mu\text{g/l}$ for most of the phenylureas, with a lowest linear range of 0–5 $\mu\text{g/l}$. The linearity was verified, with a correlation coefficient above 0.995, for all the compounds from 0.1 to 5 $\mu\text{g/l}$, with a 25 ml sample volume. Since the probability of all these compounds being together at levels of 5 $\mu\text{g/l}$ in an unknown environmental sample is very small, one can consider that there should be no limitation of recoveries by overloading the capacity with such ISs. Under the same conditions, linear ranges were obtained for all the triazines, including the dealkylated products, in the 0–3 $\mu\text{g/l}$ range.

The interest in obtaining ISs with high capacities is not for the pesticide antigen itself, but for the quantification of the other members of the class. One has to take care when speaking of the “breakthrough capacity” described by some authors who consider that the capacity is a quantity which can be simply calculated as the product of a concentration and the volume. In other words, the lower the concentration is, the

higher the sample volume can be, without loss in recoveries. This is usually verified for the pesticide antigen, but not for all the related compounds. The capacity is usually lower for related analytes than for the pesticide antigen and one has to keep in mind the fact that recovery is also dependent on the breakthrough volume which, in case of ISs, depends strongly on the affinity of the antibody for the analyte.

6.3.2.4. Validation studies

A validation study was reported which used an anti-isoproturon IS for the determination of phenylureas in water through an interlaboratory study with Aquacheck certified samples [203]. The extraction was performed with cartridges packed with 1 g of anti-isoproturon IS and the sample volume was 50 ml. Calibration curves were linear in the range between 1 and 3 $\mu\text{g/l}$ for each compound using LC-DAD. The overall relative standard deviation given by comparing the values obtained from this method and the real values given by the Aquacheck organization varied between 1 and 22%. All the samples were analyzed simultaneously by LC-DAD and LC-APCI-MS in order to confirm the identity of compounds.

Another example illustrates both the high selectivity of the immunosorbents and comparisons using alternative extraction procedures. Figure 6.23a shows the chromatogram corresponding to the analysis of a soil extract which was obtained after a classical solvent extraction, and was then diluted in pure water and analyzed on-line

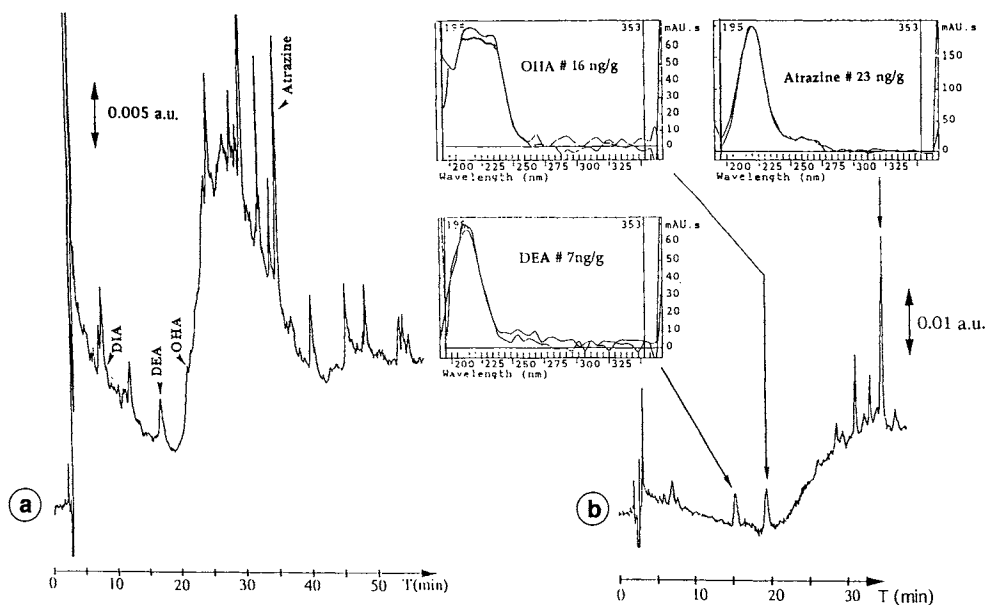


Fig. 6.23. Aqueous residue of a non-spiked soil percolated (a) onto a non-selective sorbent (apolar copolymers PLRP-S) and (b) onto anti-atrazine IS. The inserts represent the match of the UV spectra.

using a precolumn packed with a non-selective polymeric sorbent (PLRP-S). The chromatogram contains many peaks and a huge hump, but it was possible to identify atrazine, de-ethylatrazine (DEA), and hydroxyatrazine (OHA) which is only slightly visible, thereby rendering impossible any quantification unless a subsequent clean-up is performed. Figure 6.23b shows the analysis of the same extract, but using a precolumn packed with an anti-atrazine IS instead of PLRP-S. One can see the advantages of the high selectivity of the pretreatment and the easy identification and quantification of each analyte, including hydroxyatrazine which has a characteristic UV spectrum. For compounds with specific UV spectra, the identification is strongly reinforced by the selective preconcentration, the retention time, and the UV spectrum. The concentrations of DEA and atrazine were found to be similar to those obtained from GC measurements after a clean-up of the samples.

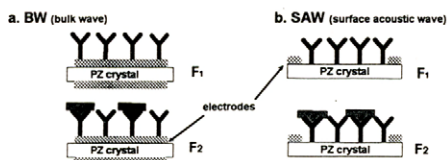
6.3.3. Further developments of immunosorbents

Class-specific immunosorbents are expected to be developed and to become available in the near future for immunochemical sample preparation, as a consequence of the wider acceptance of immunoassays by analytical chemists. The commercialization of these immunosorbents will require the availability of reproducible antibodies, which can be better guaranteed by monoclonal antibodies. Our group has compared the class-specific properties of ISs for phenylureas using monoclonal antibodies against isoproturon, atrazine and simazine. The first results indicate that higher capacities are obtained because of the higher concentration of the monoclonal antibodies. Similar recoveries have been measured for the two groups of pesticides, using similar conditions including the procedure for bonding the antibodies onto silica [204]. This is a promising result for the development of class-specific IS. Other classes, such as phenoxyacetic acids or carbamates are under development.

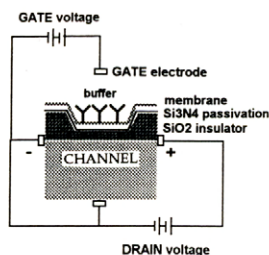
6.4. BIOSENSORS

Much effort has been made in the last decade to develop biosensors, in response to the need for fast, sensitive, cost-effective, continuous and in situ monitoring methods. Because of the different approaches taken by different research groups, the definition of a biosensor has evolved from the classical concept of an enzyme electrode to include a variety of configurations. A biosensor is defined as a miniaturized device integrating an immobilized biological sensing element attached to an appropriate transducer [5,205–208]. The sensing element can comprise enzymes, antibodies, DNA or microorganisms. The transducer may be electrochemical, optical or acoustic. Figure 6.24 gives the schematics of the main types of transducers employed in combination with immobilized antibodies as biological sensing elements. Electrochemical transducers measure changes in current or voltage, optical transducers

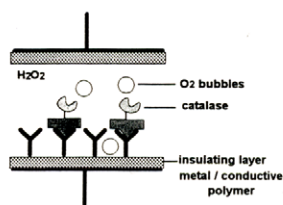
A. Piezoelectric



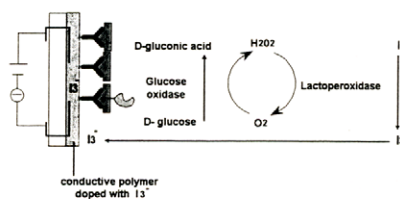
B. Potentiometric (FET)



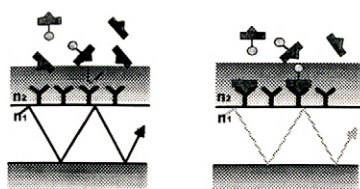
C. Capacitive



D. Conductimetric



E. Optic: EW



F. Optic: SPR

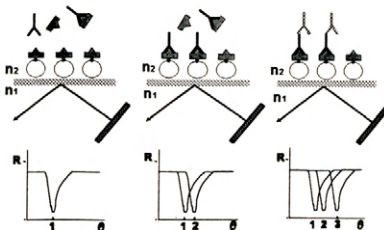


Fig. 6.24. Biosensors: schemes of the main types of transducer employed in combination with a biological sensing element (here, antibodies). From Ref. [6].

measure changes in fluorescence, absorbance or reflectance and acoustic transducers measure changes in frequency resulting from small changes in a mass bound to their surface.

Advances in biochemistry, molecular biology and immunochemistry have expanded the range of biological recognition elements whereas developments in fibre optics and micro-electronics have expanded the capabilities of signal transducers. Biosensors represent an active area of research and are beginning to move from the

laboratory concept stage to field testing and commercialization [208]. Some biosensors are already available, one example being a microbial sensor for the determination of biochemical oxygen demand (BOD) which has reduced the 5 day conventional measurement to a 15 min field determination [209,210].

A biosensor should respond directly, selectively and continuously to the presence of one or several analytes, so the biological reaction should ideally be highly reversible to provide accurate on-site, real-time measurements. However, very few biosensors meet all these requirements. Most of those reported do not give a direct answer to the presence of a contaminant because they measure a secondary signal which is the product of an enzymatic reaction, or a fluorescent compound. Some of them do not work under reversible conditions, although they include a flow-through cell, and need to be regenerated before the next measurement takes place. Others are difficult to use on-site or only allow a single use.

In this section, we present the main characteristics and give examples of the recent developments in biosensors with applications for pesticide monitoring. The different types of transducers are discussed according to the immunosensor description of Fig. 6.24.

6.4.1. Immunosensors

Immunosensors are based on the principles of solid-phase immunoassays. As with immunoassays, it is difficult to detect events which are directly derived from the antigen-antibody reaction. Most reported devices perform indirect measurements by using competitive immunoassay configurations, or labels such as enzymes, fluorescent chemicals or electrochemically active substances. Amplification of the signal thus depends on the labels. Proteins will be used as mass labels in piezo-electric immunosensors; fluorescent labels are well suited for optical immunosensors, and enzymes capable of catalyzing the formation of an electroactive compounds are required for electrochemical immunosensors.

The interest in developing immunosensors for pesticide analysis is based on the technology that exists now and the possibility of obtaining specific antibodies for almost any pesticide. However, a severe limitation of immunosensors is that antigen-antibody interactions are not readily reversible, in contrast to most enzyme biosensors where there is a catalytic reaction. Therefore, the immunosensors reported to date are generally irreversible, single-use or regenerable devices. Because of the high affinity constant of the immunoreaction, regeneration of the sensing layer can be troublesome. It can be achieved either by displacing the immunoreaction equilibrium, or by disrupting the analyte-antibody reaction with organic solvents, alone or in combination with acidic buffers or with a chaotropic agent, or by using low affinity antibodies [211–215].

Immunosensors are usually classified according to the type of transducer associated with the sensing antibodies.

6.4.1.1. Piezoelectric sensors

Piezoelectrics are materials that can be brought into resonance by the application of an external alternating electric field. Quartz crystals are common example. The frequency of the resulting oscillation is determined by the mass of the crystal. By coating a piezoelectric with antibodies one obtains immunosensors which can, in principle, be used for the direct detection of the antibody–antigen reaction, owing to a mass increase. The signal can be enhanced in a competitive immunoassay where the analyte and mass-labelled (as a protein) analyte derivative compete for antibody sites immobilized on the quartz surface. There are two modes for setting up piezoelectric immunosensors (see Fig. 6.24A).

In bulk wave acoustic (BW) devices, adsorption of the analytes occurs on the surface, but resonance occurs within the entire mass of the crystal. The surface is connected to an oscillator circuit, and when the crystal is in contact with a sample containing the analyte–antigen, the mass of the crystal will increase and the resonance frequency will decrease according to the Sauerbrey equation: $\Delta f = -2.3 \times 10^6 f^2 (\Delta m/A)$, where f is the oscillation frequency in Hz, Δm is the mass of the adsorbed material in g, and A is the sensing area in cm^2 .

In surface acoustic wave (SAW) devices, an acoustic wave moves just at the surface of the crystal (Fig. 6.24A). Mass loading on the acoustic path between two sets of electrodes will alter the phase surface wave velocity and generate a shift in the frequency.

The limitations of this technique are the lack of specificity and the possible interferences produced when the devices are used in aqueous media [5]. One advantage is the low cost of the instrumentation required. Applications have been reported for the detection of atrazine [216–218], 2,4-D [219] and parathion [220]. Table 6.13 reports the main characteristics of these piezoelectric immunosensors. The greatest sensitivity has been obtained using a label immunoreactant [217]. Protein A has been used to orientate the antibodies on the crystal adequately [218].

Two commercial devices exist for preparing piezoelectric immunosensors. One contains a liquid-flow cell and a computer program to make real-time assays of the antibody–antigen interaction (PZ Immunobiosensor System from Universal Sensors Inc., New-Orleans, LA, USA) and the other is designed for simultaneous electrochemical and weight measurements using a dip or a well holder (EG&G, Princeton Applied Research, New Jersey, USA).

6.4.1.2. Electrochemical biosensors

Because of its simplicity, electrochemical transduction coupled to enzymes permits the formation of low-cost biosensors. However, electrochemical detection of an immunoreaction is difficult and enzymes which lead to the formation of electroactive substances are necessary. The corresponding immunosensor configurations are often known as electrochemical immunoassays [221].

Amperometric immunosensors measure the current generated by oxidation or re-

TABLE 6.13
FEATURES OF THE REPORTED IMMUNOSENSING DEVICES FOR PESTICIDES (ADAPTED
FROM REFS. [5,205])

Transducer types	Analytes	Competitive IA Y/N	Immobilized reactive	Labelled reactive	Detection limit ($\mu\text{g/l}$)	Ref.
Piezoelectric						
	Atrazine	N	Ab-protein A	No	0.03	[218]
	Atrazine	Y	Hapten	No	0.1	[216]
	Atrazine	Y	Ab	Hapten-protein	0.01	[217]
	2,4-D	Y	Hapten	No	1	[219]
	Parathion	N	Ab	No	35	[220]
Electrochemical						
	2,4-D	Y	Ab	Hapten-HRP	40	[225]
	2,4-D	Y	Ab	Hapten-HRP	1	[227]
	2,4-D	Y	Hapten	Ab-HRP	0.1	[222,223]
	2,4,5-T	Y	Ab	Hapten-HRP	50	[226]
	Atrazine	Y	Ab	Hapten	0.025	[229]
Optical (EW and SPR)						
	Terbutryn	Y	Hapten	Fluorescein-Ab	0.1	[235]
	Atrazine	Y	Ab	Fluorescein-hapten	2–5 nM	[214]
	Atrazine	Y	Ab	Fluorescein-hapten	0.1	[234]
	Atrazine	Y	Hapten	No	0.05	[239]
	Parathion	Y	Hapten-casein	Fluorescein-antiIgG	0.3	[238]
	Imazethapyr	Y	Ab	Fluorescein-hapten	0.3	[236,237]

duction of redox species at an electrode surface which is maintained at an appropriate electric potential. The size of current observed has a linear relationship with the concentration of the electroactive species. The electrode is usually constructed of platinum, gold or carbon. The immunoreactive substance of a competitive immunoassay is immobilized directly onto the surface electrode, or entrapped by a membrane adjacent to the electrode. The enzymes used as labels in the immunosensors are usually oxido-reductases (such as horseradish peroxidase, HRP) or hydrolytic enzymes (such as alkaline phosphatase, AP) that yield an electroactive species as a product of the enzymatic reaction. A mediator is often required in order to avoid an electrochemical response from some other substances present in the sample.

Very few examples of applications have been presented up to date. A liposome immunosensing device adapted for single-use field immunoassays has been described, for determining alachlor and PCBs. In these assays, competition takes place between the free analyte and the analyte conjugated to liposomes containing either a dye, such as sulforhodamine B for an optical detection, or an electroactive substance such as potassium ferrocyanide that can be detected amperometrically [186,187]. A disposable amperometric immunosensor permitted the determination of 2,4-dichloro-

phenoxyacetic acid (2,4-D) with detection limits close to $0.1 \mu\text{g/l}$ [222,223]. Another device for measuring 2,4-D has been reported which combines the use of the antibodies to extract the analyte selectively from the sample and subsequently measure it using an immobilized acetylcholinesterase electrode [224].

Potentiometric immunosensors detect the change in potential that occurs after specific binding of the antibody to the antigen. Antibodies in aqueous solutions are polyelectrolyte proteins and consequently their electrical charge can be affected when binding the corresponding antigen. The potential difference is measured between the working electrode, where the specific antibody has been immobilized, and a reference electrode. A disadvantage of this arrangement is the very small variation of potential (1–5 mV), because the immunoreaction leads to poor sensitivity, and because of limitations by background effects. One example was reported to determine 2,4-D by grafting antibodies to a graphite electrode using electrodeposition [225]. The immunoelectrode was dipped into a mixture containing buffer, 2,4-D, and 2,4-D-HRP conjugate for 5 min and, after rapid washing, was dipped into an electrochemical cell containing H_2O_2 in a buffer and the substrate. The system could be regenerated and the detection limit was $40 \mu\text{g/l}$. A similar device allowed the determination of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) down to $50 \mu\text{g/l}$ [226].

Silicon field effect sensors are based on semi-conductor technology and can be constructed either as capacitors or as field effect transistors (FETs). In these devices, the conductivity of the channel region (measured by the application of a voltage between source and drain electrodes) is controlled by the strength of the electrical field generated by a gate, providing amplification. ImmunoFET devices have one of the immunoreactive species placed on the gate electrode (see Fig. 6.24B). Miniaturization of these sensors on a chip and integration into a complete automated system is easily feasible. A portable pH-sensitive FET device has been developed to determine 2,4-D down to $1 \mu\text{g/l}$ [227]. Potentiometric detection takes place after a pH change occurring as a result of peroxidase oxidation of ascorbic acid using *o*-phenylenediamine as an electron mediator.

Capacitive immunosensors are shown schematically in Fig. 6.24C and are based on the fact that the capacitance of electrolytic capacitors depends on the thickness and dielectric behaviour of a dielectric layer placed on the surface of a metal plate. Changes in the dielectric constant which result from antibody–antigen interactions are thus measured. Bresler et al. [228] of Biotronic System Inc. have developed a prototype that is based on the biochemical generation of bubbles. Oxygen bubbles are generated by a catalase reacting with H_2O_2 as the label agent of an ELISA system and result in a change in capacitance. This system has been applied for glucose determination. It could be applicable to small analytes by coating different antibodies onto the surface of this prototype.

Conductimetric and impedimetric sensors involve the measurement of the disturbance of a sensing layer placed between two electrodes, as with capacitive sensors (Fig. 6.24D). Sandberg et al. have developed a device to measure atrazine, based on

the use of an electroconductive polymer doped with iodine, to which specific antibodies had been immobilized [229]. This sensor uses a direct competitive immunoassay where I_3^- is formed by enzymatic reactions and incorporated into the polymer, producing a change in its conductimetric properties. The detection limit is as low as $0.025 \mu\text{g/l}$. A portable electrode-based immunoassay was also developed, called SmartSense™ (Ohmicron Corporation), which takes place within 15 min and is designed to be used in the field for the determination of atrazine [230].

6.4.1.3. Optical immunosensors

Optical sensors are based on the absorption or emission of light by the immunoreactants. These types of sensors take advantage of the low cost and flexibility of optical fibres.

Fibre optic biosensors (FOBs) consist of a fibre-optic strand having an appropriate sensing layer on the distal tip of the fibre. Light is introduced into the proximal end and travels to the distal tip by total internal reflection. Changes in the absorbance, luminescence, polarization, or refractive index can all be used as optical transduction mechanisms [231,232]. The simplest approach to an optical detection system uses the measurement of the fluorescence emission from one of the immunoreactants involved in the reaction. As an example, benzo[a]pyrene bound to antibodies immobilized on the distal part of a fibre optic can be detected, owing to its intrinsic fluorescence when excited by laser radiation transmitted into the fibre, with a detection limit of a few femtomoles [233]. However, only a few analytes can be detected directly from their intrinsic fluorescence. Most analytes have a characteristic wavelength maximum of absorbance, but the absorbed light is only a small percentage of the total transmitted. This fact explains why competitive immunoassay configurations using fluorescent labels are preferred.

Evanescent wave (EW) immunosensors have been developed for pesticide applications in recent years. When light is propagated through a waveguide (n_1 , see Fig. 6.24E) by multiple total internal reflections, an electromagnetic wave called an evanescent wave is generated in the optically rarer external medium (n_2 with $n_2 > n_1$). Since the distance of penetration of the evanescent wave is only a few hundred nanometres, only surface-bound molecules will interact with the light. The principal advantage of this system is that it avoids possible interferences from the bulk media. When molecules having an absorption spectrum which includes the excitation wavelength are located in the evanescent field they absorb energy, leading to an attenuation in the reflection light of the waveguide. This measurement is known as attenuated total reflection (ATR). However, the analyte must have a characteristic absorption band and the absorbed light is usually a very small percentage of the total transmitted light, leading to poor sensitivity. To increase the sensitivity, it is necessary to make use of labelled molecules that can re-emit the absorbed evanescent photons at a longer wavelength, as fluorescence. Part of this emission is coupled back to the waveguide and is transmitted to the receptor. This phenomenon is known as total internal reflection fluorescence (TIRF) and is the basic principle of most im-

munosensors reported to detect environmental pollutants with sufficient sensitivity. It was applied to the detection of atrazine [214,234], terbutryn [235], imazethapir [236,237] and parathion [238], with detection limits at the low 0.1 $\mu\text{g/l}$ level. Most of the devices make use of fluorescein as label.

Surface plasmon resonance (SPR) immunosensors contain an optical electronic transducer. A surface plasmon is an evanescent electromagnetic field generated at the surface of a metal conductor (usually Ag or Au) when excited by the impact of light of an appropriate wavelength at a particular angle. Electrons at the metal surface are excited by the incident light, producing a resonance at a frequency different from that of the electrons in the bulk of the metal film (see Fig. 6.24F). The absorption of light energy during resonance is observed as a sharp minimum in light reflectance when the varying angle reaches a critical value, which depends on the wavelength and polarization of the incident light and on the dielectric properties of the medium adjacent to the metal surface. Therefore, the critical angle value is affected by analytes binding to the surface and can allow the monitoring of biological interactions. A commercial apparatus, BIAcore™ (Pharmacia), available for coating with the desired antibodies or antigens, was used to develop a SPR-based immunosensor for atrazine detection [239]. Another system called IAsys™ (Fisons) was developed to monitor binding events in real time [240]. It exploits a novel form of optical biosensor that combines the technology of waveguides with the SPR phenomena, the metal layer being replaced by a dielectric resonant layer of high refractive index (i.e., titania or zirconia) and separated from the glass prism by a low-refractive index layer of silica.

Reflectometric interference spectroscopy (RIFS) immunosensors have also been described as optical immunosensors. The basic principle uses the reflected light produced when a light beam passes through the interface between two media of different refractive index. A thin transparent film will produce an array of reflected beams at each of the interfaces, which can be considered as only two reflected beams when the reflectance of the interfaces is small. The phase difference of these beams is directly related to the thickness of the layer, and therefore changes in the film thickness can be determined by changes in the interference spectrum. Brecht et al. applied this principle to make an immunosensor which can detect atrazine using a hapten derivatized immunosensor surface, without any label, with detection limits around 0.25 $\mu\text{g/l}$ [241].

6.4.2. Enzyme biosensors

Glucose was the first substrate to be detected by enzyme biosensors [242]. In the biosensors described so far, the detection of pesticides is not based on their enzymatic transformation but on their inhibiting capacity on a specific enzyme reaction.

The target enzymes of pesticides are usually well known, but not all are commercially available, or they may be hard to purify or unstable. Marty et al. have recently

reviewed the potential of enzyme biosensors for pesticide analysis [243,244]. Esterases or oxidative enzymes are most frequently used as sensing elements, in combination with electrochemical transducers, although the use of fibre optics has also been reported.

Cholinesterases (acetylcholinesterase and butylcholinesterase), inhibited by organophosphorus, and carbamate pesticides are used most widely because they are commercially available in a high degree of purity and stability. Their specific activity can be monitored by electrochemical methods. Potentiometric transducers such as the ion-selective electrode (pH electrode), and ion-selective field effect transistor have been described [245–247]. Inhibition of acetylcholinesterase has also been measured using a light-addressable potentiometric sensor [248]. Various amperometric transducers are employed which use the cathodic reduction of oxygen (Clark electrode), or the anodic oxidation of hydrogen peroxide or thiocholine [249–256]. One enzyme fibre-optic biosensor was developed for the detection of anticholinesterase compounds [257].

A comparison of the performances of cholinesterase sensors is difficult because of the different standards and techniques applied. According to the review by Marty et al. [243], the sensitivity depends on the type of cholinesterase and the source. As an example, the limits of detection for carbaryl were measured as 19 and 250 $\mu\text{g/l}$ using acetylcholinesterase from the electric eel and butylcholinesterase from horse serum, respectively, whereas the detection limits of paraoxon were 12 and 1.5 $\mu\text{g/l}$, respectively, with the same enzymes [254]. The limits of detection obtained for some insecticides (azinphos-ethyl, malathion, parathion, parathion-methyl) and their “oxon forms”, which differ by chemical substitution of the sulfur in the thioester group by an oxygen, are strongly different for biosensors using the same enzyme (cholinesterase from the electric eel) and comparable experiments [243]. For the “oxon forms”, typical limits of detection are in the range 0.5–15 $\mu\text{g/l}$, whereas the limits of detection of the native insecticides were above 1000 $\mu\text{g/l}$. Therefore, it seems better to oxidize the samples before analysis in order to increase the sensitivity of the biosensors.

Amperometric detection has been shown to be the most sensitive detection method, as shown in Table 6.14, although electroactive species can interfere in the measurement.

As a first indication of the performance of one enzymatic sensor described above, validation studies have been carried out using environmental samples [244,258]. The biosensor consisted of a two-electrode system, comprising a platinum electrode with immobilized cholinesterase and a commercial Ag/AgCl electrode connected to an amperometric detector [259]. For this purpose, two batches of freeze-dried samples containing carbamate and organophosphorous pesticides were studied using a cholinesterase-based biosensor and an on-line solid-phase extraction, liquid chromatography, and diode-array detection (SPE-LC-DAD) procedure. Enzyme inhibition by the pesticides was studied in aqueous media using a flow injection analysis (FIA)

TABLE 6.14

LIMITS OF DETECTION OF SOME CARBAMATE AND ORGANOPHOSPHORUS INSECTICIDES: COMPARISON OF POTENTIOMETRIC AND AMPEROMETRIC TRANSDUCTION (FROM REF. [243])

Insecticides	Limits of detection ($\mu\text{g/l}$)	
	Potentiometric	Amperometric (thiocholine oxidation)
Aldicarb	1140	1.9
Carbaryl	1000	19
Carbofuran	6	0.02
Dichlorvos	300	22

configuration or in organic solvent, supposed to be representative of analysis after an off-line solid-phase extraction of the water samples. The results are reported in Table 6.15. Although the results obtained with FIA are expressed in nM and in paraoxon-equivalent, whereas the results from SPE-LC-DAD are expressed in $\mu\text{g/l}$ for each pesticide and for their total, there is some agreement between results of the two batches. Batch B exhibits the higher results, either using the on-line SPE-LC-DAD system or the biosensor, both FIA and the organic solvent system, indicating that the sensor has permitted the distinction between two samples with a difference of 2.6-fold. The detected levels are levels of alarm in river water samples and are also found in river and estuarine waters during the first days after the application of pesticides [260]. This indicates that the biosensor system can be used as a pre-screening method for the organophosphorus pesticides analyzed and for carbaryl, and can differentiate between pesticide concentrations of 3.9 and 10.3 $\mu\text{g/l}$. However, no agreement was found between the results obtained with the FIA and after use of off-line SPE in organic samples. The discrepancy between the systems could be partly explained by the known behaviour of organophosphorus pesticides and the biosensor used. The biosensor is specially sensitive to the oxo-metabolite and the whole FIA system needed approximately 1–2 h for complete analysis of the water samples. This period is sufficient to degrade some of the organophosphorus pesticides, mainly by abiotic hydrolysis and photolysis [260].

Different immobilizing strategies have been used for the determination of pesticides which inhibit the hydrolysis of acetylcholine, resulting in a reduction in the measured potentiometric signal in a light-addressable potentiometric sensor [248]. Paraoxon and bendiocarb were detected at levels around 10 nM, whereas higher concentrations were required for some other organophosphorus pesticides, aldicarb, and methomyl.

Oxidases include tyrosinase, lactase, peroxidase, and aldehyde dehydrogenase. Tyrosinase has been used for catalysis of the ortho hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones whereas molecular oxygen is reduced

TABLE 6.15

CONCENTRATION OF PESTICIDES FOUND IN TWO BATCHES OF FREEZE DRIED WATER SAMPLES ANALYZED BY A CHROMATOGRAPHIC METHOD AND BY A CHOLINESTERASE-BASED BIOSENSOR (FROM REF. [258])

Method	Compounds	Batch A	Batch B
SPE-LC-DAD ^a	Fenamiphos	1.26 ± 0.04	5.78 ± 0.07
	Carbaryl	0.97 ± 0.11	1.26 ± 0.01
	Fenitrothion	0.19 ± 0.01	0.14 ± 0.01
	Parathion-ethyl	1.49 ± 0.10	3.18 ± 0.02
	Total	3.91	10.36
Biosensor ^b	FIA configuration	2.76 ± 0.11	17.72 ± 0.98
	Organic system	0.53 ± 0.04	7.92 ± 0.51

Water samples were freeze-dried and stored at -20°C.

^aConcentration in µg/l.

^bConcentration expressed in nM as equivalent of paraoxon. "FIA configuration" corresponds to analyses performed on aqueous media and "organic system" corresponds to analyses performed with organic solvent.

to water. This enzyme has been successfully used in amperometric sensors with solid graphite and carbon paste electrodes for the determination of phenolic compounds [261–264]. The method starts with an enzyme's catalytic cycle and then the incorporation of enzyme electrodes into simple flow injection or integrated sample -handling units. Phenolic compounds can be detected accurately down to the sub-µl levels in river water.

The ability of various pesticides, such as atrazine, dithiocarbamates and hydrazines, to inhibit tyrosinase has also been studied [265–269]. With these sensors, the actual detection limits are of the order of µM, but the sensitivity can probably be increased. Dithiocarbamate fungicides have been measured by their ability to inhibit the enzyme, aldehyde dehydrogenase. As an example, maneb can be detected at concentrations close to 0.05 g/l by measuring the reduction in the current generated by the oxidation of propionaldehyde to the corresponding carboxylic acid [270].

6.4.3. Microbial sensors

Microbial sensors use living organisms, such as bacteria, yeast and fungi as sensing elements. They exploit the metabolic functions of these microorganisms to effect the detection and measurement of pollutants. In fact, they are derived from bioassays which are based on several principles such as the transformation rate of carbon, nitrogen or sulfur, enzyme activity, growth, mortality, oxygen consumption or luminescence [205,209,271].

Microbial sensors can be considered to offer a form of bioassay in which the

microorganism is combined with an appropriate transducer. Different transducers have been combined with microorganisms. Electrochemical biosensors usually consist of a membrane containing the immobilized microorganism in contact with an electrochemical device. Either the consumption of oxygen or the appearance/disappearance of an electrochemically active metabolite is measured. Such biosensors have been developed for monitoring anionic surfactants such as linear alkylbenzenesulfonates (LAS), by measuring the respiration activity of immobilized LAS-degrading bacteria, which have been isolated from activated sludge [272]. Some electrochemical microbial sensors have been reported to screen mutagenicity, using *Salmonella typhimurium*, *Escherichia coli* or *Bacillus subtilis* [205]. Other electrochemical micro-scale biosensors have been developed for monitoring nitrate, and atmospheric gases such as CO₂, NO₂, NH₃ and methane [205,273], PCBs [274], phenol [275,276], chlorinated phenolic compounds [277,278], sulfide [255], and biochemical oxygen demands in organic-polluted water [209,210,279].

Because many herbicides target photosynthesis, several photosystem-based sensors have been developed [243]. Various biological receptors have been used, such as prokaryotic cells [280,281], unicellular algae [282], spinach chloroplast membranes [283], thylakoid membranes [284], and reaction centres isolated from bacteria [285,286]. The herbicide inhibitory effect is usually detected electrochemically or by light absorbance. Photosystem-based biosensors have been described for the detection of atrazine, with detection limits in the range 10–260 µg/l, depending on the sensing element [280,282,284,285]. Other photosystem-based sensors have been developed for the detection of phenylurea herbicides such as chlortoluron [284], diuron [281], isoproturon [282], linuron and metoxuron [280], and for the detection of propanil, ioxynil and bromoxynil [280,282]. However, organelles, isolated membranes, and reaction centres require complex preparation are often poorly stable [243]. Cyanobacterial and algal cells have the advantage of being easily maintained in pure culture and being more stable when immobilized.

Other photo-microbial sensors exploit the luminescence produced by naturally occurring photobacteria or other genetically modified microorganisms [205]. This principle has been used for the development of sensors for the detection of the biochemical oxygen demand [287], or to measure a toxicity equivalent such as in the Microtox test (Beckman Instrument). No application to pesticides has been reported to date.

6.5. CONCLUSIONS AND PERSPECTIVES

The area of environmental chemical analysis and monitoring is expanding worldwide. Field analytical methods have been shown to reduce the time required and the cost of environmental monitoring. Immunochemical methods and biosensors will certainly be further developed in the near future as simple, cheap, and efficient methods for monitoring pesticides (and other pollutants) in the environment.

Immunoassays are now at an advanced stage compared to biosensors. There is an increasing availability of immunoassays, developed for monitoring many pesticides. However, the situation is different in North America, where immunoassays are now accepted as a valuable analytical method, from that in Europe, where they still have to be accepted and validated. The dipstick format will certainly be the “field format” of the near future.

The first IA developments dealt with single pesticides, but there is clearly a trend for developing class-specific immunoassays, especially for the monitoring of herbicides in water, because several active ingredients are applied to the same areas and detected together. For the same reason, the simultaneous detection of a plurality of analytes by immunochemical techniques would answer many of the requirements of pesticide pollution monitoring. In this direction, Brecht and Abuknesha [288] have reported that recent advances in non-environmental target application areas indicate that immunochemically based simultaneous multi-analysis capabilities are possible, with an emphasis on simplicity, avoidance of sample treatment, speed, sensitivity, automation, and low cost.

Although enzyme-based, antibody-based, and microbial biosensors have been reported for the detection of pesticides, these methods are just moving from the laboratory stage to field-testing and commercialization. However, as evidenced by the number of reported biosensors for pesticides, it appears probable that biosensors will be among the future tools for monitoring purposes. Although some of them display high sensitivity, stability, and portability, the biosensors developed hitherto for pesticide analysis do not meet all the required criteria. To be successful and to compete for field screening applications with immunoassay kits, there are a number of significant improvements to be made before they can be used under current regulations. For performing continuous monitoring, there is much competition with other well-established and more widely accepted methods. Biosensors will have to fulfil the same requirements as conventional robust techniques, i.e., acceptable short- and long-term reproducibility, absence of false negatives above the quantification limits, and sufficient robustness when they are applied to a variety of matrices [205].

Future advances in the development of immunochemical methods and biosensors will require scientists of different disciplines to combine their research efforts. Improvements in the transducer technology should permit direct detection on environmental samples and provide the necessary flexibility for field analytical methods. Antibody-based biosensors may increase the number of compounds than can be analyzed, as a result of the progress that recombinant DNA technology is now making to this field. Immunosensors have the capability of detecting single compounds or a group of compounds. Enzyme-based biosensors seem better addressed to developing class-specific devices. Newly discovered microorganisms such as hyperthermophilic bacteria, gene engineering techniques, and new synthetic biomaterials may also bring new developments for microbial sensors [209].

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